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# Surgical Forum

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# *Foreword*

Each year an increasing number of recently completed research projects are submitted to the Forum Committee for consideration for the annual Forum on Fundamental Surgical Problems. Judging from the abstracts submitted, I am convinced that they are increasing in excellence as well as in number. They represent a wide variety of basic investigations in general surgery and in the surgical specialties. From these abstracts the Committee is able to select a program of unsurpassed quality. This volume contains brief, carefully written and illustrated descriptions of the research efforts presented at the annual Forum. It is a volume that should be in the hands of all those who are interested in current progress in surgery.

These papers are representative of the best which is being accomplished by young American surgeons. They constitute a tribute to the growing interest, enthusiasm and investigative capacity of these young surgeons. They reflect the steadily increasing availability of proper research facilities, both laboratory and clinical. They were made possible by research funds from government agencies, private foundations and local institutions. This wholesome state of affairs speaks well for the future of surgery.

The members of the Forum Committee express their appreciation to Helene Coleman for her excellent work in editing the manuscripts included in Volume VII of the SURGICAL FORUM.

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## OBSTETRICS AND GYNECOLOGY



## NEUROSURGERY AND NEUROPHYSIOLOGY

## TUMOR PROBLEMS AND TRANSPLANTATION

## PLASTIC SURGERY



## UROLOGY

## ORTHOPEDICS

*Shock*

# TOLERANCE OF DOGS RESUSCITATED WITH DEXTRAN TO SUBSEQUENT SURGICAL STRESS*

GEOFFREY SLANEY

Experiences in the Korean war proved beyond doubt the value of Dextran as a resuscitative agent when used in quantities of the order of 1000 cc to 1,500 cc.[1,2] Experimentally, it has been shown that Dextran will adequately resuscitate animals which have undergone severe blood loss of 40 to 50 per cent of the blood volume.[3,4] As far as can be ascertained, however, no experimental work has been done on how well animals thus resuscitated withstand subsequent major surgery. As this may have important clinical implications, this research was undertaken to study the response of animals to a major operative procedure following severe blood loss corrected by Dextran replacement.

## METHOD

Sixty-nine dogs were used as the experimental subjects and they were divided into 2 groups. Group 1 consisted of 19 dogs and group 2 of 50 dogs. All experiments were performed under morphine and nembutal anesthesia.

Group 1 consisted of 9 dogs which served as control animals and 10 dogs which were the experimental animals. The control animals were bled 40 to 50 cc per kg of body weight, this blood being collected in a heparinized container and later retransfused back into the animals. The period of hypotension following bleeding averaged 5 minutes or so, during which the blood pressure was 20 to 40 mm Hg. When retransfusion had been completed, a stress operation consisting of cholecystectomy, splenectomy and left nephrectomy was then performed. Exactly the same procedure was followed in the experimental animals except that the blood withdrawn was replaced by an equal volume of 6 per cent Dextran in 0.9 per cent saline.

Group 2 consisted of 25 control dogs and 25 Dextran dogs. The same procedure was followed as in group 1 but the stress was considerably increased by bleeding all the animals a further 15 cc per kg of body weight (blood not replaced) after the operation had been completed.

## RESULTS

The group 1 experiments were designed to simulate patients who had undergone massive Dextran replacement and then had to undergo a major operative procedure without further blood loss. The mortality rate in this group was 20 per cent in the Dextran resuscitated dogs as compared with 0 per cent in the control animals.

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* From the Department of Surgery, University of Illinois College of Medicine, Research and Educational Hospital, Chicago, Illinois. Supported in part by a grant from the Graduate School of the University of Illinois.

The Dextran used was supplied by Abbott Laboratories.

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The group 2 experiments were designed to simulate clinical conditions where following a massive Dextran replacement a patient might have to undergo subsequent major surgery accompanied by further frank or latent blood loss Among the control dogs in this group the mortality rate was 4 per cent, whereas in the Dextran dogs it was 72 per cent The experimental animals in this group were unable to tolerate the final bleeding procedure whereas the control animals could do so In the Dextran dogs the immediate postoperative blood pressure was satisfactory but fell precipitously following the final hemorrhage and complete circulatory collapse ensued The tolerance of these animals to hypotension following the initial hemorrhage was also reduced as compared with that of the controls

The results of this study were expressed as mortality rates in each group and were as follows

| Group 1 Bleeding + Stress Operation | | Mortality Rate |
|---|---|---|
| 9 Control dogs | Blood replaced | 0% |
| 10 Dextran dogs | Blood replaced with Dextran | 20% |

| Group 2 Bleeding + Stress Operation + Additional Bleeding | | Mortality Rate |
|---|---|---|
| 25 Control dogs | Blood replaced | 4% |
| 25 Dextran dogs | Blood replaced with Dextran | 72% |

## DISCUSSION

After operation all animals that survived recovered consciousness within 8 hours and many achieved this state within 2 hours With 2 exceptions all animals that did not recover within 8 hours were already dead or were in established progressive peripheral circulatory failure and died shortly afterwards All the non survivors died within 16 hours of the operation In the main the Dextran dogs took longer to return to consciousness than the dogs which received blood Constant low grade capillary oozing from the abdominal incision was the usual finding in the vast majority of the dogs which had received Dextran and there can be no doubt that these animals had an impairment of the blood coagulation mechanism Usually the oozing from the incision had ceased by the time the wound was closed but this was not invariably the case

The autopsies on the Dextran dogs which died showed essentially similar findings There was a variable quantity of blood stained fluid in the peritoneal cavity the quantity roughly corresponded to that found in control animals sacrificed at a similar time postoperatively The stomach small bowel and colon were ashen white virtually bloodless and the musculature strongly contracted but in no case examined was there any evidence of hemorrhage into the bowel lumen or necrosis of the mucosa The liver was extremely pale and shrunken in size The remaining kidney was examined in approximately one third of the cases and apart from its extreme pallor appeared microscopically normal No evidence of infarction was seen as reported by Pirani and his associates[3] The thoracic viscera all appeared normal apart from pallor and the usual terminal congestion of the lower lobe of the dependent lung

## CONCLUSIONS

1 The tolerance of dogs resuscitated with Dextran to subsequent surgical stress is lower than that of dogs resuscitated with blood

2 Animals resuscitated with Dextran and undergoing subsequent surgery will not tolerate further blood loss and should this occur, additional replacement should be with blood and not with more Dextran

3 Animals resuscitated with Dextran have a much reduced tolerance to hypotension as compared with animals resuscitated with blood

4 There is a definite hemostatic defect after infusions of large volumes of Dextran Despite investigations by numerous workers the nature of the defect remains obscure and awaits further elucidation

5 For hemorrhage of moderate degree Dextran is very effective as a blood substitute

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# A SIMPLIFIED METHOD FOR BLOOD VOLUME DETERMINATIONS USING RADIOACTIVE ISOTOPES*

WILLIAM A SPENCER J RICHARD THISTLETHWAITE AND SOLOMON N ALBERT

Our interest in the physiological variations in blood volume experienced during anesthesia and surgery prompted our efforts toward the development of a simple technique for liquid phase counting adaptable to both the laboratory and the operating room

A plastic coil was developed for use with a well type scintillation counter The coil is prepared from Clay Adams PE 260 polyethylene tubing internal diameter 07 inches The tubing is wound around a glass rod and inserted into the well counter The introduction of the coil has efficiently replaced the standard aliquot measuring techniques

## METHOD

CR [1] tagged red blood cells may be used with the coil The patient s own blood is tagged or a stock preparation of O Rh negative tagged cells is used

From the Departments of Anesthesiology and Surgery District of Columbia General Hospital and George Washington University Hospital Washington D C From the Anesthesia and Surgery Research Laboratory District of Columbia General Hospital Supported in part by the U S Atomic Energy Commission Contract AT (30 1) 1820

The stock cells are prepared by adding 1 mc of sodium radiochromate (R) to 500 ml of whole blood in 150 ml A C D solution Ten to 20 ml of cell pack is removed from the stock solution and washed twice before being re suspended in normal saline to make a total volume of 25 to 30 ml

When determining blood volume the cell suspension of standard is counted in the coil at a pre set time Twenty milliliters of this suspension containing approximately 15 to 20 microcuries is administered to the patient Allowing 15 minutes for adequate mixing a sample is withdrawn from the patient and counted through the coil at the same pre set time used for the standard The patient s blood volume is then calculated by the following formula

$$\frac{\text{counts of standard} \times \#\ \text{ml administered}}{\text{counts from patient} - \text{background}} = \text{total blood volume}$$

Between each determination of standard and dilute sample the coil is washed with 10 ml of normal saline solution and the background count is rechecked

Since there is no adherence of activity to the coil repeated determinations are possible This merely requires determining the patient s background before the administration of subsequent doses of standard The formula is then modified as follows

$$\frac{\text{counts of standard} \times \#\ \text{ml administered}}{\text{counts of patient} - \text{patient background}} = \text{total blood volume}$$

Serial determinations may be carried out with as many doses of the standard as required being given as long as the patient s retained activity does not exceed 250 microcuries

The technique is the same for RISA with the exception that a 1 150 or 1 200 dilution of the standard RISA solution is prepared in plasma or whole blood This was found to be necessary as the concentrated RISA solution adheres to the coil raising the background counts in spite of repeated washings

RESULTS

Comparing this method for blood volume determinations with the standard aliquot methods *in vitro* we have found the volume determinations through the coils to be more accurate A comparison between diluted and undiluted standard is presented as an example (See Table I ) For more complete statistical data reference is made to the original report of this technique [1]

*Table I Volume Determinations*

Comparison between diluted and undiluted standards calculated from total activity

| Measured vol in ml | Undiluted std Coil | Undiluted std Aliquot | Diluted std Coil | Diluted std Aliquot |
|---|---|---|---|---|
| 3530 | 3501 | 3385 | 3510 | 3478 |
| 3550 | 3546 | 3448 | 3553 | 3506 |
| 3570 | 3549 | 3116 | 3558 | 3543 |

There is no reduction in the accuracy of the volume determinations when the coil is subjected to variations in temperature from 15 to 10°C or to pressure changes up to 150 mm Hg

The administration of 20 ml volumes of cell suspension brings about a more even distribution in a shorter period of time than does the administration of the equivalent isotope dosage in 10 ml volume or less (See Fig 1)

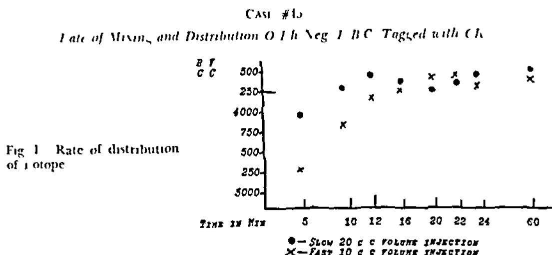



Fig 1 Rate of distribution of isotope

DISCUSSION

With the introduction of the coil we feel the technique of blood volume determinations has been greatly simplified Utilizing the constant volume obtained with the non variable coil and a pre set time for counting all samples has eliminated several mathematical steps

Further simplification is manifested by a marked reduction in the technical errors encountered with the previously described methods, since our technique requires a minimum of glassware and eliminates transferring the activated sample from test tube to test tube by means of a pipette The samples may be drawn directly into the coil from a catheter or needle in the femoral vein (see Fig 2) or may be introduced directly from the syringe used to remove the blood sample through the coil without detailed meas

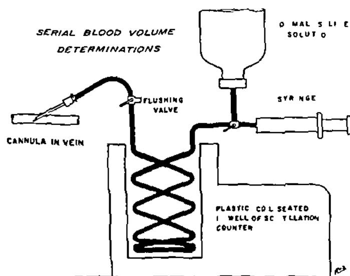



Fig 2 Diagrammatic representation of plastic coil being used with well counter for blood volume determinations

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Fig 1. The experimental set up. The dog bleeds into a limp plastic bag enclosed in a water filled tank. The blood displaces an equal amount of water into an open cylinder. Sensing electrodes connected to an electronic control maintain the water surface always at 47.4 cm above the aorta of the dog regardless of the bleeding volume by means of a reversible motor which can raise or lower the cylinder. Changes in bleeding volume are recorded on a moving kymograph by means of a pulley system connected to the cylinder. By adjustment of clamps at the end of the experiment water is pumped back into the tank with the syringe. This forces blood back into the dog through the filter.

donor dog was readied with polyvinyl catheters in the aorta and inferior vena cava inserted *via* the left femoral artery and vein. A third polyvinyl catheter was placed in the right femoral artery of the donor dog to measure blood pressure. All cannulations in both experimental and donor dogs were done using local piperocaine anesthesia. Two hours following sedation the experimental dog was heparinized using 2 mg/kg intravenously and hemorrhaged to 35 mm/Hg mean arterial pressure utilizing a new automatic *leveling device and blood reservoir (Fig 1). Following attainment of maxi*mal bleeding volume at 35 mm Hg (1.15±.48 hr in 60 previous hemorrhage experiments) the superior mesenteric artery of the shocked dog was isolated through a left flank retroperitoneal approach. The artery was clamped off from the aorta and cannulated. The profound shock plus small amounts of local piperocaine anesthesia was ample for this brief procedure. Following cannulation controlled cross perfusion of the artery (double Sigmamotor pump) was immediately started at a rate of 8 to 10 ml/kg/min utilizing arterial blood from the donor. (See Fig 1.) Equal amounts of vena caval blood were returned from the perfused dog to the normotensive donor dog also heparinized 2 mg/kg. The shocked dog's mean superior mesenteric arterial pressure was maintained at about 110 mm Hg with this flow. With the use of the automatic leveling device the perfused dog's mean systemic arterial pressure was maintained at 35 mm Hg throughout the period of perfusion. When the perfused dog had been in shock 1½ hours the perfusion was stopped, the cannula removed from the superior mesenteric artery and the artery quickly repaired with 6-0 silk. The flank wound was tightly closed

urements This makes the technique applicable to the operating room for serial blood volume determinations during surgery yet within the realm of those having little technical knowledge regarding isotopes

At the present time William Swan M S working in our laboratory is assembling a dilution meter computer which will give direct blood volume readings

SUMMARY

1 A plastic coil simplifying the technique of liquid phase counting is presented

2 Both $CR^{51}$ labeled red cells and radioactive iodinated human serum albumen may be used with the coil

3 Errors of manipulation and transfer are curtailed contamination is reduced and the method is adaptable for serial blood volume determinations in the operating room

REFERENCE

1 Albert S N Spencer Wm A Finkelstein M Alpert S and Coakley C.S A plastic coil simplifying radioactive liquid phase counting In press J Laborat Clin M 1956

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## THE PREVENTION OF IRREVERSIBLE HEMORRHAGIC SHOCK IN DOGS BY CONTROLLED CROSS PERFUSION OF THE SUPERIOR MESENTERIC ARTERY*

RICHARD C LILLEHEI

The primary lesions of irreversible hemorrhagic shock in the dog are severe mucosal congestion and necrosis in the small[1] and large bowel [2] During prolonged hemorrhagic shock the superior mesenteric venous flow reflecting superior mesenteric arterial flow drops to less than 25 per cent of normal In order to study the effect on irreversibility of preventing these pathological changes a procedure was developed to maintain the superior mesenteric arterial flow of unanesthetized shocked dogs at normal levels

METHOD

All experiments were done in an air conditioned room and utilized unanesthetized mongrel dogs sedated with morphine sulfate 2 mg /kg intramuscularly Prior to hemorrhage polyvinyl catheters were placed in the superior and inferior vena cavae of the dog to be hemorrhaged *via* the external jugular and femoral veins Another polyvinyl catheter was placed in the femoral artery for bleed out and reinfusion An unanesthetized sedated

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*Table 1 Hemorrhagic Shock at 35 mm. Hg Mean Arterial Blood Pressure**

| TYPE OF PERFUSION | NO. DOGS | NO. OF SURVIVORS | BODY WEIGHT KG. | MAXIMAL BLEEDING VOLUME: BODY WEIGHT | MAXIMAL BLEEDING VOLUME: BLOOD VOLUME† | PERFUSION: START IN HOURS AFTER HEMORRHAGE | PERFUSION: FLOW ML./KG./MIN. | HOURS IN SHOCK | HOURS TO DEATH AFTER SHOCK |
|---|---|---|---|---|---|---|---|---|---|
| Superior mesenteric arterial | 30 | 27 | 19.7±3.3 | 6.0 ±.91 | 58.6±7.6 | 1.18±.38 | 8.81±2.2 | 1.90±.21 | |
| Superior mesenteric arterial—Eck fistula dogs | 10 | 9 | 16.1±3.6 | 5.09±.39 | 51.5±3.9 | 1.01±.28 | 9.52±2.3 | 5.0 ±.20 | |
| None | 15 | 1 | 20.0±5.1 | 5.73±.55 | 53.5±4.6 | | | 1.40±.40 | 6.39±5.9 |
| Aortic | 10 | 2 | 17.5±2.1 | 5.78±.64 | 57.3±5.6 | 1.32±.19 | 10.9 ±2.1 | 1.61±.20 | 7.03±6.1 |
| Inferior vena caval | 10 | 2 | 20.1±1.9 | 6.01±.93 | 56.0±1.5 | 1.35±.07 | 9.0 ±.92 | 1.91±.29 | 5.86±3.1 |

*Mean values with standard deviations.
†Blood volumes were not corrected for plasma trapping in the hematocrit tube or for body hematocrit.

and the dog was reinfused with all shed blood in the reservoir about 5 hours following induction of hemorrhage

Control dogs similarly sedated and heparinized were hemorrhaged to 35 mm Hg and the superior mesenteric artery exposed but not cannulated One group of control dogs received no perfusion one group received aortic perfusion *via* the femoral artery and a third group inferior vena caval perfusion *via* the femoral vein during the shock All control perfusions utilized the same rate of flow per kg of body weight as was used in superior mesenteric arterial perfusions

To rule out the beneficial effect on the liver of increased portal blood flow in superior mesenteric arterial perfusions chronic Eck fistula dogs were similarly shocked and received superior mesenteric arterial perfusion as described above The portacaval shunts were of the end to side type

Blood volumes were measured on all dogs before and after the experiment with a micromethod using T 1824 [3] Clean surgical technique was utilized in all experiments All surfaces exposed to blood were of non wettable plastic and were soaked in benzalkonium chloride for 24 hours before use

## RESULTS

Table I briefly summarizes the results Dogs receiving aortic or vena caval perfusion or no perfusion were in shock for somewhat less than 5 hours because they generally showed signs of excessive uptake and/or cardiorespiratory failure necessitating reinfusion before the prescribed period was completed Dogs were not considered survivors unless they were alive at the end of 72 hours following the experiment. Dogs dying following the hemorrhagic shock manifested the usual signs of irreversibility They had a recovery of blood pressure to normal levels followed by gradual decline with the onset of copious bloody diarrhea At autopsy these dogs exhibited severe mucosal congestion and necrosis of the small and large bowel along with marked liver congestion Surviving superior mesenteric arterial perfused dogs sacrificed at varying periods following the experiment showed normal bowels The liver of these dogs however showed the same degree of congestion both grossly and microscopically as was seen in controls dying of irreversible hemorrhagic shock

Because of the accuracy of the Sigmamotor pump it was not necessary to give the donor dogs any extra blood during the procedure Moreover blood volume studies done before and after experiments showed no significant blood volume changes in perfused dogs There was no donor mortality due to irreversible shock after any of these experiments

## DISCUSSION

There are several reports of prevention of irreversibility by perfusion of the liver of dogs during hemorrhagic shock [4, 5, 6, 7] All such perfusion experiments have utilized anesthetized dogs with the exception of those of Frank *et al* [4] (1946) Moreover these investigators have started perfusion almost coincident with hemorrhage a fact which markedly alters the maximal bleeding volume They have also used a considerably shorter period of shock than was used in the present studies These factors make the comparison of liver perfusion with bowel perfusion *via* the superior mesenteric artery very difficult However Edwards *et al* [8] (1954) showed that occlusion

*Table 1  Hemorrhagic Shock at 55 mm Hg Mean Arterial Blood Pressure**

| TYPE OF PERFUSION | NO DOGS | NO. OF SURVIVORS | BODY WEIGHT KG | MAXIMAL BLEEDING VOLUME: BODY WEIGHT | MAXIMAL BLEEDING VOLUME: % BLOOD VOLUME † | PERFUSION: START IN HOURS AFTER HEMORRHAGE | PERFUSION: FLOW ML/KG/MIN | HOURS IN SHOCK | HOURS TO DEATH AFTER SHOCK |
|---|---|---|---|---|---|---|---|---|---|
| Superior mesenteric arterial | 30 | 27 | 19 7±3 3 | 6 0 ±.94 | 58 6±7 6 | 1 48±.38 | 8 84±2.2 | 4 90±.23 | |
| Superior mesenteric arterial—Eck fistula dogs | 10 | 9 | 16 1±3 6 | 5 09±.39 | 51 [illegible]±3 9 | 1 01±.28 | 9 52±2 3 | [illegible] 0 ±.20 | |
| None | 15 | 1 | 20 0±5 1 | 5 73±.55 | 53.5±4 6 | | | 1 10±.10 | 6 39±3 9 |
| Aortic | 10 | 2 | 17 5±2 4 | 5 78±.61 | 57 3±5 6 | 1 32±.19 | 10 9 ±2 4 | 4 64±.20 | 7 03±6 1 |
| Inferior vena caval | 10 | 2 | 20 4±4 9 | 6 04±.93 | 56 0±4 5 | 1 35±.07 | 9 0 ±.92 | 4 94±.29 | [illegible]86±3 1 |

*Mean values with standard deviations

†Blood volumes were not corrected for plasma trapping in the hematocrit tube or for body hematocrit

of the aorta for 2 hours above the superior mesenteric artery but below the celiac axis resulted in a higher mortality in dogs than occlusion just below the superior mesenteric artery with simultaneous hepatic artery occlusion Parkins *et al*[9] (1955) reported that differential intestinal cooling during a 2 hour occlusion of the thoracic aorta prevented death of dogs, whereas differential cooling of the liver did not

Heretofore the congestion seen in the bowel of dogs dying of irreversible hemorrhagic shock has generally been attributed to hepatic vasoconstriction with resultant portal congestion Yet Frank *et al*[10] (1951) showed that chronic Eck fistula dogs were no more resistant to irreversible hemorrhagic shock than normal dogs These experiments on Eck fistula dogs were duplicated by the author At autopsy however, contrary to Frank *et al*[10] almost the same degree of mucosal congestion and necrosis of the bowel was seen in these dogs as was seen in normal dogs dying of irreversible hemorrhagic shock The marked mucosal necrosis present as well as the congestion has not been fully appreciated by many investigators This necrosis apparently is the result of critical ischemia occurring during the shock period possibly enhanced by the rich bacterial flora present Superior mesenteric arterial perfusion during hemorrhagic shock prevents these changes and prevents irreversibility from occurring Moreover the survival of chronic Eck fistula dogs similarly perfused rules out increased portal flow to the liver as the basis for survival

If bowel necrosis is the cause of irreversibility following prolonged hemorrhagic shock then there is a close resemblance between this condition and experimental bowel obstruction in dogs Indeed the same abnormal hemoglobin pigment found by Nemir *et al*[11] (1919) in the peritoneal fluid and plasma of dogs dying of strangulation obstruction has been found by the author in the plasma of dogs dying of irreversible hemorrhagic shock It is not present in the plasma of those dogs perfused through the superior mesenteric artery during the hemorrhagic shock

## SUMMARY

Irreversibility has been prevented in 90 per cent of a group of 30 dogs by perfusing the bowel *via* the superior mesenteric artery during hemorrhagic shock Results were identical in a group of 10 chronic Eck fistula dogs Bowel pathology is strongly suggested as a cause of irreversibility following prolonged hemorrhagic shock

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# A STUDY OF MITOCHONDRIAL CHANGES IN HEMORRHAGIC SHOCK*

J G STRAWITZ AND H HIFT

The advent of transfusions of unlimited quantities of blood ushered in a great advance in the prevention of death due to hemorrhage However it has become apparent in recent wars and in civilian practice that there is still a significant number of patients suffering from prolonged periods of hemorrhagic hypotension who are refractory to blood transfusion therapy These patients show the signs and symptoms of the condition known as irreversible hemorrhagic shock

It is the purpose of this study to describe some of the intracellular morphological and biochemical changes which appear to accompany irreversible hemorrhagic shock Several studies[1,2] involving tissue analyses reported data which suggested that the energy metabolism of the tissues might be a site of injury This focused our attention on the mitochondria since it had been shown in recent years that these subcellular particles are responsible for the oxidative and energy converting functions of all living cells

## METHOD

*Hemorrhagic shock was produced in dogs by a modification of the Wiggers* technique The methods used in preparing formalin fixed heart mitochondria and examining them under phase contrast or isolating them fresh in sucrose suspension and determining their enzymatic activities have been described elsewhere[3] The formation of crescents was observed in mitochondrial suspensions which were diluted in sucrose solutions of suitable compositions allowed to stand at room temperature for varying periods of time fixed with neutral 10 per cent formaldehyde and counted under oil immersion in a Spencer phase contrast microscope For spectrophotometric analysis the mitochondrial suspensions were diluted 1 5 with water incubated at 37°C over night and centrifuged The clear supernatants were examined in the Beckman DU spectrophotometer

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*From the Department of Surgery and Cardiovascular Research Laboratory University of Wisconsin Medical School Madison This investigation was supported by the Wisconsin Heart Association by the Department of the Army Office of the Surgeon General and by the National Heart Institute of the National Institutes of Health U S Public Health Service

With the technical assistance of Hackchan Rhee

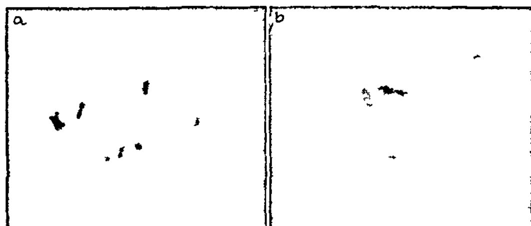



Fig 1 Heart mitochondria from normal and shocked dogs prepared in formaldehyde and examined under phase contrast
a — normal b — shock
Magnification app 2300 x

RESULTS

***In Situ* Morphology** *Figure 1 shows free floating mitochondria which were* separated from the myofibrils by blenderization in formaldehyde In the undisturbed tissue long chains of mitochondria are arranged longitudinally on all sides of the myofibrils The free floating normal particles tend to be square to spindle shaped rods with distinct boundaries and dense to moderately translucent centers The particles observed in preparations from shocked dog hearts are swollen irregular in shape with indistinct boundaries and translucent centers Blenderates from brain liver and kidney also show abnormal mitochondria

**Behavior in Sucrose Suspension** Harman[4] made the observation that mammalian mitochondria from various sources when allowed to stand in sucrose suspensions underwent a series of morphological alterations the normal rods rounded off into solid spheres which swelled and ballooned and finally formed large signet ring shaped forms which he called crescents (Fig 2) He found that these transformations occurred more readily under

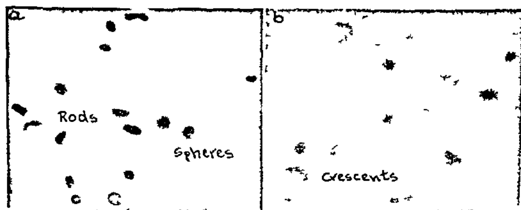
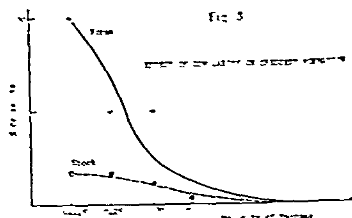
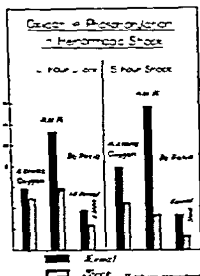
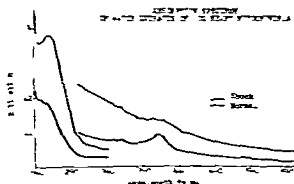



Fig 2 Heart mitochondria from a normal dog isolated by differential centrifugation suspended in 0.5 M sucrose and examined under phase contrast
a — fresh b — after standing at room temperature
Magnification app 2300 x




such environmental conditions as low tonicity and elevated temperatures In our hands normal dog heart mitochondria yielded large numbers of "crescents" in sucrose solutions of low molarity at temperatures above freezing and at neutral pH the yields increased with time In every instance the mitochondria obtained from the paired shocked animal underwent this transformation much less readily and the "crescents" which formed tended to be smaller and more compact than in the normal (Fig 3)

The water extracts derived from the mitochondria of shocked dogs generally contained more protein than similar normal preparations and had higher extinctions especially in the flavin and U V regions (purine and pyrimidine-containing material) (Fig 4)

As described elsewhere the oxidation of Krebs cycle intermediates and of fatty acids was essentially unimpaired even after 5 hours of shock No requirement for coenzymes could be demonstrated. However in the presence of the hexokinase system oxygen uptakes and phosphate esterification were often decreased (Fig 5)

### DISCUSSION

The results thus far indicate that heart mitochondria are structurally altered in irreversible hemorrhagic shock resulting in changes in size and distortion of shape Simultaneously their oxidative phosphorylation mechanism appears to be affected They seem to be less responsive to environmental conditions since they form crescents less readily than the normal At the same time they apparently are more water soluble i e they either contain more water soluble material or their internal substance has been altered so as to render it more soluble At this point it is difficult to formulate a hypothesis which would account for these abnormal behavior patterns although one may be inclined to look upon them as expressions of one specific intramitochondrial defect which is caused by prolonged hemorrhagic hypotension

### SUMMARY

Heart mitochondria from dogs subjected to irreversible hemorrhagic shock differed in size and shape from those of normal dogs They tended to show lowered P/O ratios in Warburg experiments formed crescents less readily under a variety of environmental conditions and yielded water extracts which contained higher concentrations of protein flavins and purine and pyrimidine containing material than did similar preparations from normal dogs

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## THE RELATIONSHIP OF BLOOD VOLUME TO EXCHANGEABLE POTASSIUM AN INQUIRY INTO THE CONCEPT OF CHRONIC SHOCK *

H VICTOR PARKER JAMES D MCMURREY AND FRANCIS D MOORE

The nature and significance of the biochemical changes characteristic of cachexia are being elucidated by studies of body composition [1] These studies show that in the absence of severe chronic hemorrhage patients suffering from starvation cachexia quite characteristically have a slightly enlarged blood volume expressed in terms of observed weight and due largely to an expansion of the plasma fraction

*From the Department of Surgery and Laboratories for Surgical Research of the Peter Bent Brigham Hospital and Harvard Medical School Boston Massachusetts This work was sponsored by the Subcommittee on Metabolism in Trauma Advisory Committee on Metabolism Office of the Surgeon General Department of the Army and supported in part by the Atomic Energy Commission

Various therapeutic measures designed as supportive in these states are essentially empirical or based on concepts not in accord with the characteristic compositional defects as they are understood today There is widespread acceptance of the principle of massive transfusion of whole blood in these patients preoperatively under a concept of chronic shock relating to a markedly reduced blood volume[2] Clark *et al* have described blood volumes (dye hematocrit method) in patients with cachexia of a large variety of types with correction of deficits by administration of 1500 ml to 1000 ml of whole blood In addition there is some adherence to the suggestion of giving voluminous quantities of plasma to effect a favorable protein state[3] Volumes suggested are in the range of 2500 to 7000 ml in the period preceding surgery

This paper attempts to define more closely this compositional disturbance relating to the vascular components and to assess its significance in chronic illness

## SELECTION OF CASES

The observations are associated with a larger study of total body composition by simultaneous dilutional techniques first outlined at this Forum in 1955[1,4] One hundred and twenty five such studies have now been carried out.

As the majority of our cases have been chronically ill some aspect of depletion is usually present However this orientation considers a group in which cachexia secondary to a disease state has been the prominent clinical picture without distortion by an associated condition such as heart failure renal disease or ascites Twenty five such studies are available for selection Factors such as duration of weight loss over one year the presence of associated bleeding of recently administered transfusion of indirectly related electrolyte imbalance and the administration of cortisone further exclude the use of certain cases

The findings in 8 males and 5 females suffering cachexia as determined by history of weight loss and clinical appearance are presented The group encompasses a variety of disease entities and includes such surgical conditions as ulcer carcinoma regional ileitis ulcerative colitis and sepsis The average weight loss was 12 1 kg or 19 per cent of body weight with a range of 3 1 to 19 2 kg or from 5 to 34 per cent of body weight None of these patients had detectable bleeding of a degree to warrant their exclusion none had recent transfusion none were edematous all had histories under one year s duration and there were no other apparent complicating factors

## RESULTS

Normal values are based on a series of 20 total compositions carried out in these laboratories and on values in the literature Data selected from the measured and derived values in each composition study for this analysis are plasma volume red cell volume total blood volume total exchangeable potassium and intracellular water expressed in terms of per cent of normal

The mean large vessel hematocrit was 87 per cent of normal with a range of 75 to 100 per cent The mean whole body hematocrit was 76 per cent of normal with a range of 53 to 86 per cent.

The distribution of the individual values of the blood volume in relation to the exchangeable potassium is presented in Figure 1

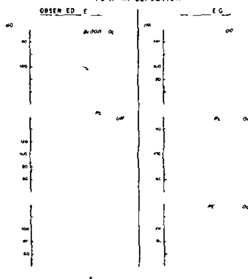



Fig 1 The distribution of the individual values of the blood volume in relation to exchangeable potassium The shaded areas represent the exchangeable potassium values against which the plotted individual values for blood volume plasma volume and red cell volume are to be compared It will be noted that the red cell mass is reduced proportionately to the K but that plasma volume (and to a lesser extent blood volume) is significantly higher

Total blood volumes scatter in the range above 100 per cent of normal in terms of observed weight and although slightly below the normal range for pre illness weight in neither instance are as low percentage wise as the exchangeable potassium data Plasma volumes show a wide scatter above normal for observed weight but distribute themselves in a normal pattern for the patient s normal weight Again they do not coincide with the range of exchangeable potassium the lowering of the latter being extensive (as a result of tissue wasting) while the intravascular volumes are significantly higher The red cell volumes distribute in the range of the exchangeable potassium values in both instances

The relation of the arithmetic mean and standard deviation of the entities together with the values for intracellular water is portrayed in Figure 2

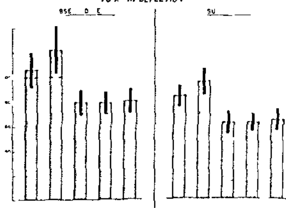



Fig 2 The relation of the arithmetic means and standard deviations of the blood volume plasma volume red cell volume exchangeable potassium and intracellular water Again as in Fig 1 the RV is proportional to K and ICW whereas PV is disproportionately high

This summarizes the changes in more graphic form The mean plasma vol ume is 122 per cent of normal with a range of 96 to 162 per cent of normal for observed weight 97 per cent of normal with a range of 89 to 113 per cent for usual weight The mean total blood volume is 106 per cent of normal for the observed weight with a range of 83 to 130 per cent and 85 per cent of normal with a range of 76 to 99 per cent when expressed in terms of usual weight The red cell volume in the case of observed weight has a mean value of 79 per cent with a range of 63 to 97 per cent of normal the exchangeable potassium is 79 per cent with a range of 67 to 93 per cent the intracellular water 81 per cent with a range of 67 to 96 per cent

For usual weight the mean red cell volume is 63 per cent of normal with a range of 44 to 77 per cent the mean exchangeable potassium value is 63 per cent of normal with a range of 49 to 73 per cent the intracellular water 65 per cent of normal with a range of 48 to 76 per cent

## DISCUSSION

Total exchangeable potassium representing approximately 95 per cent of the total body content of potassium is directly proportional to the lean body mass As such its reduction represents a significant index of wasting in the depleted patient This premise relating to cell wasting is substantiated by the correlation found in reduction of the independently obtained value for intracellular water (total body water minus corrected bromide volume) This correlation extends to the intravascular cellular equivalent—the red cell vol ume On the other hand simultaneously measured plasma volume reveals a completely independent expansion of this extracellular phase resulting in a blood volume somewhat above normal for the observed weight

Expression of these indices in terms of weight in usual health shows an ex tremely poor correlation between reduction in total blood volume and ex changeable potassium and in addition gives values for plasma volume in the range of normal The three intracellular entities show parallel defects

## CONCLUSIONS

The selection of patients as outlined in the initial section of the paper is emphasized If chronic hemorrhage plays a role in the wasting disease significant blood volume reductions may be observed Similarly long stand ing depletion and the presence of edema secondary to extremely severe de pletion will influence results

In conclusion in this selected group of chronically debilitated patients we have been unable to confirm the presence of a described syndrome character ized by consistently and often drastically reduced whole blood volumes and thus correctable by whole blood transfusion Similarly on the basis of ex panded plasma volume and expanded extracellular fluid space (these are relative expansions only) the use of large quantities of plasma in these pa tients should be approached with caution

There is little clinical doubt concerning the need for accurate intravascu lar replacement in the preoperative period in patients with hemorrhage acute or chronic we are not concerned with the problem here However in discriminate preoperative administration of whole blood based on the picture of chronic cachexia alone will inevitably result in serious overloading of the circulation in many patients The use of separated cells would seem war

ranted The approach to infusion therapy requires increasing assessment in terms of selected components[5]

These conclusions direct attention to other fields of interest the relation of the serum albumins to the tissue proteins and closer definition of need for intravenous albumin therapy the role of whole body hematocrits osmolarity effects and other influences in interpretation of the hematocrit and other common clinical concentration values (The suggestion that they are invalid because of reduced plasma volume does not seem warranted) In addition the significance of the parallel reduction of red cell mass with lean tissue protein should be noted in assessing priority concepts

SUMMARY

1 Selected measurements from the body composition studies of 13 patients suffering from uncomplicated depletion are presented

2 The trend of intravascular change in depletion is presented as a reduction of red cell volume paralleling the reduction in lean tissue mass the indices for the latter being exchangeable potassium and intracellular water The absolute plasma volume alters little and thus there is partial preservation of the total blood volume

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## CIRCULATORY RESPONSES TO INTRA ABDOMINAL MANIPULATION DURING SURGERY*

A G ROCCO AND L D VANDAM

Pronounced changes in the circulation have long been observed with intra abdominal manipulation during surgery[1 2 3] In a 1 year period at our hospital 92 or 17 5 per cent of 526 patients undergoing laparotomy exhibited noticeable falls in blood pressure Changes in the pulse rate were less obvious but bradycardia was not an unusual finding The nature of these circulatory responses has been studied further in 68 patients undergoing ab

* Peter Bent Brigham Hospital Boston Massachusetts

dominal operations of whom 38 were women and 30 men All categories of physical status were represented The anesthetics given and the types of operations performed may be seen in Table I The anesthetics were given intratracheally in most cases by anesthetists of varying experience and ether was the usual anesthetic agent Most of the cases were in the upper abdomen with cholecystectomy predominating

*Table I Anesthetics Given and Operations Performed During Study of Abdominal Reflexes*

| ANESTHETICS | NUMBER |
|---|---|
| Nitrous Oxide Ether and Combinations with Other Agents | 34 |
| Cyclopropane and Combinations with Other Agents | 15 |
| Spinal Anesthesia and Other Conduction Anesthetics | 13 |
| Nitrous Oxide Pentothal and Curare | 4 |
| General Anesthesia and Hypothermia | 2 |
| TOTAL | 68 |

| OPERATIONS | NUMBER |
|---|---|
| Gall Bladder and Common Duct | 31 |
| Gastrectomy | 3 |
| Other Upper Abdominal Operations | 6 |
| Abdominal Vascular Surgery | 6 |
| Lower Abdominal (Gynecologic and Bowel) | 22 |
| TOTAL | 68 |

METHOD

In all patients continuous observation of the brachial arterial blood pressure was made by means of a Statham P23D strain gauge attached to an 18 gauge intra arterial needle and recorded on a direct writing Sanborn twin viso electronic recorder Mean arterial blood pressures were obtained electronically in most cases and in a few by planimetric integration under the arterial curve Electrocardiograms were taken in 50 patients Since the possibility existed that changes in the circulation might be secondary to alterations in intrathoracic pressure respiratory activity also was followed by recording esophageal pressure A plastic catheter such as is ordinarily used for nasal administration of oxygen was passed into the esophagus connected to a Statham P23D strain gauge and the pressures recorded alternately with the electrocardiogram in 12 cases

Another explanation for the development of arterial hypotension lay in the possibility of increasing depth of anesthesia as surgical exploration was carried out At this time a maximum depth is needed To make certain of a constancy of anesthetic level electroencephalographic monitoring of the level of anesthesia was carried out in 25 cases with fronto occipital leads on a Grass electroencephalograph

The aforementioned *recordings were started early in the course* of anesthesia Correlations were made between changes in the records and activity in the surgical field When the peritoneal cavity was entered a deliberate series of maneuvers was carried out to ascertain the effective stimuli responsible for development of the expected circulatory changes Accordingly the

ranted The approach to infusion therapy requires increasing assessment in terms of selected components[5]

These conclusions direct attention to other fields of interest the relation of the serum albumins to the tissue proteins and closer definition of need for intravenous albumin therapy the role of whole body hematocrits osmolarity effects and other influences in interpretation of the hematocrit and other common clinical concentration values (The suggestion that they are invalid because of reduced plasma volume does not seem warranted) In addition the significance of the parallel reduction of red cell mass with lean tissue protein should be noted in assessing priority concepts

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2 The trend of intravascular change in depletion is presented as a reduction of red cell volume paralleling the reduction in lean tissue mass the indices for the latter being exchangeable potassium and intracellular water The absolute plasma volume alters little and thus there is partial preservation of the total blood volume

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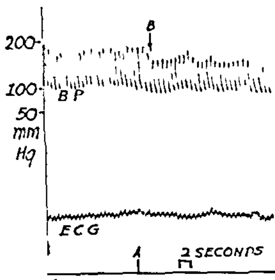



Fig. 2. Continuous arterial blood pressure and electrocardiographic recordings in a 70 year old woman undergoing cholecystectomy and common bile duct exploration with nitrous oxide ether and curare. At A a hand was inserted in the abdominal cavity. Blood pressure fell within 2 seconds and continued to fall in a stepwise manner. There was no change in the electrocardiogram.

ment of the liver, insertion of the hand into the peritoneal cavity and movement from one area to another, placement of packing, evisceration and retraction of the wound edges produced the change. Deliberate traction on the mesenteries of bowel and stomach failed to produce hypotension in several cases. Likewise, stimulation of the celiac plexus was ineffective. Clamping the gallbladder wall and traction failed, but exploration in the common duct region produced a marked response even with care taken to avoid obstruction of the portal and caval veins. In 8 patients, when the vena cava was occluded, there was no effect in 1 and minimal changes in the others.

Analysis of the pulse wave contour provided some clue as to the circulatory adjustments involved. In Figure 3 it can be seen that during hypotension there is a flattening of the wave suggesting a decrease in cardiac output rather than a decrease in peripheral resistance. The change in output could be a manifestation of lesser venous filling or decreased cardiac contractility. Additional circulatory measurements are required to establish the specific nature of the changes.

The electrocardiographic changes observed in lead II were by no means as frequent or uniform as the changes in blood pressure. Of the 50 patients studied, 17 exhibited auriculoventricular dissociation with nodal rhythm or nodal rhythm alone. These abnormalities were associated with a variety

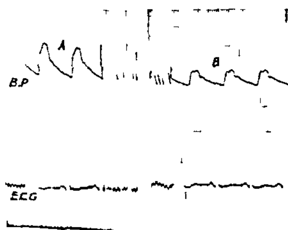



Fig. 3. Tracings from the same patient as in Figure 2 show pressure pulse contours before (A) and after (B) production of hypotension by intra-abdominal manipulation. Paper speed has been changed to illustrate the change more clearly. The decrease in pulse pressure occurred without change in the duration of systole or the position of the dicrotic notch. This suggests a decrease in cardiac output rather than change in peripheral resistance.

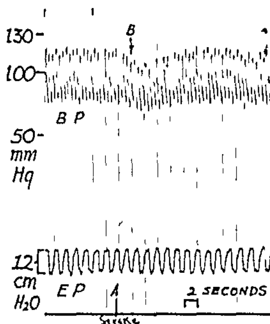



Fig. 1. Continuous arterial blood pressure (upper tracing) and esophageal pressure (lower tracing) in a 52 year old woman undergoing cholecystectomy with nitrous oxide and ether. At A the parietal peritoneum of the upper anterior abdominal wall was stroked with a small ball sponge. Within 2 seconds, at B, systolic and diastolic pressures fell. The maximum fall was 15 mm. Hg systolic and 5 mm. Hg diastolic. The duration of the hypotension was 8 seconds. There was no change in esophageal pressure.

surgeon rubbed localized areas of the parietal and visceral peritoneum with a small ball sponge as discretely as possible. Friction on the mesenteries, pressure over the area of the coeliac plexus, traction on the gallbladder and stomach, and occlusion of the portal and caval veins were the other stimuli applied.

## RESULTS

In 55 of the 68 patients studied, arterial hypotension was observed in relation to abdominal manipulation. In only 1 was hypertension seen. The latter occurred during upper abdominal exploration in a patient undergoing a pelvic operation under spinal anesthesia. Eleven patients showed a negligible drop in blood pressure. In the remainder, or 44 patients, hypotension was moderate to marked. The manner in which hypotension was produced is interesting. In some cases the change seemed to be clearly reflex in origin. That is, with fairly specific manipulation and little likelihood of interference with venous return to the heart and with no change in the depth of anesthesia or accompanying respiratory phenomena, the blood pressure began to fall within a matter of seconds. A typical response may be seen in Figure 1. The maximum fall was 15 mm. Hg systolic and 5 mm. Hg diastolic. The pressure began to return before the stimulation was stopped. The fall in pressure lasted 8 seconds. A response of this type occurred with application of towels to the peritoneal margins in the incision, or with insertion of the surgeon's hand into the abdominal cavity. In some individuals these stimuli failed to produce the effect. Repeating the stimulus in another area reproduced the same response. The most sensitive area in this regard was the anterior parietal peritoneum of the upper abdomen, whereas the surfaces of the liver, stomach and other viscera were insensitive.

In other cases, although a reflex fall in blood pressure seemed likely, contributing factors could not be eliminated. The fall in blood pressure was of greater magnitude, with less tendency to revert to normal. A stepwise drop in pressure shown in Figure 2 was detected in some of the records. Displace-

# A SIMPLE METHOD FOR THE PRETRANSFUSION REMOVAL OF POTASSIUM FROM STORED WHOLE BLOOD*

*With observations on the safety of cation exchange collected blood for massive transfusions*

Roy H Clauss Hillary Chollet Salvatore J Giannini and Alexander Henderson

The desirability of a simple and inexpensive method of removing excess potassium ions from stored whole blood prior to the transfusion of large quantities is implicit in the high concentrations of potassium known to be present in whole blood stored for several weeks This study was begun to determine how readily potassium may be removed by passing whole blood through an ion exchange column as the blood is being transfused

Potassium is removed by incorporating a commercially available ion-exchange column† into the route of transfusion distal to the filter Blood may flow directly into the patient or it may be stored in reservoirs (see Fig 1) for subsequent rapid transfusion

## METHOD

**Part I** The plasma concentrations of potassium sodium and calcium were determined before and after the passage of fresh blood through the ion exchange column Human blood stored in ACD solution for varying periods of time was studied similarly Additional quantities of potassium as the chloride were added to blood and to water to note the potassium removing capacity of a single column Then ACD whole blood was passed to observe

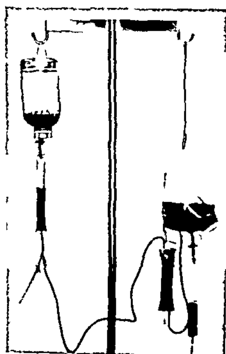


Fig 1 The ion exchange column blood pack is placed distal to a blood filter in the route of transfusion

*From the Surgical Service New York Veterans Administration Hospital and the Department of Pathology Bellevue Hospital New York

†Manufactured by Fenwal Laboratories 47 Mellen Street Framingham Mass

of factors in most cases, namely deeper levels of anesthesia and abdominal manipulation but almost all with arterial hypotension Diethyl ether was the common anesthetic agent Twenty two patients evidenced a slowing of the pulse rate 27 showed no change and 6 responded with an increase A decrease in the amplitude of the T wave and elevation or depression of the ST segment were seen in 25 instances in association with manipulation and hypotension or at other times in the course of anesthesia and operation In only 1 instance was the change pronounced enough to suggest myocardial ischemia

### DISCUSSION AND SUMMARY

The common response to intra abdominal manipulation in these clinical studies was arterial hypotension often associated with bradycardia The impression gained is that tissue deformation was the basic stimulus for this response that the parietal peritoneum was far more sensitive than the visceral and that of the upper abdomen particularly so At the present time we can only speculate about the receptors, the pathways involved and the factors leading to the circulatory alteration A possible receptor is the Pacinian corpuscle The afferent pathway might be the intercostal nerves as suggested by Reeve in work on respiration [4] Further work in laboratory animals and man will be carried out to assay the role of the phrenic nerve splanchnics, and vagus nerve in the carriage of afferent impulses from the abdomen It seems likely that the vagus nerve provides the efferent limb of the reflex arc and that vagal action alone might be responsible for the hypotension observed The occurrence of bradycardia suggests this and Peterson has recently suggested that the vagi may influence ventricular contraction

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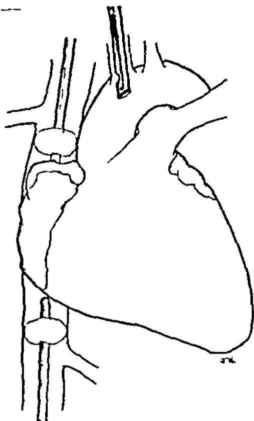


Fig 2 Balloon catheters permit occlusion of venous return to the heart without thoracotomy

RESULTS

**Part II** All dogs survived The control clotting times of 3 minutes rose to 7 to 9 minutes after the experiment The blood pressure returned to normal after each transfusion upon the deflation of the venae cavae balloons The dogs serum electrolytes immediately after the experiments averaged potasssium 2 9 sodium 149 and calcium 4 0 mEq /L One hour post experiment values were 3 2 150 and 4 5 and 2 hour concentrations were 3 2 150 and 5 5 mEq /L respectively

DISCUSSION

In 1950 C W Walter introduced to the Surgical Forum the sulfonated polystrene copolymer ion exchange column as the basis of a technique for the collection storage and administration of unadulterated whole blood[6] We have used that resin column in these experiments The original communication placed emphasis upon the decalcifying action of the resin column thereby rendering blood incoagulable without the addition of substances with detrimental physiological actions notably citrate Investigators subsequently have employed ion exchange collected blood in experiments but have added magnesium and potassium before transfusing blood in volumes comparable to the large volumes in the current experiments While the addition of chemicals to reconstitute normal ion concentrations is reasonable since it may preclude some ion deficiency side-effects this has been shown to be unnecessary for survival There is a rapid return of plasma electrolyte concentrations toward normal values to the asymptomatic range

Ivy[4] in 1943 Bruneau and Graham[2] in 1943 and Cookson[3] in 1951 published the results of dog bleeding and retransfusion experiments All dem

the volume that would flow through one column without adverse mechanical or osmotic effects. Finally, the physiologic effects of the transfusion of large volumes of blood collected *via* the ion exchange column were studied by the biologic responses of 25 dogs.

Blood samples were centrifuged and decanted. The serum or plasma samples were analyzed for sodium and potassium by flame photometry. Calcium was determined by a permanganate method modified after Roe. Fresh whole blood was collected *via* the column; aliquots were labelled with radiochromate and were reinfused into the donor and into a compatible recipient. The erythrocyte survivals were compared with the normal slope of degradation.

Osmotic fragility of ion column collected red cells was tested.

## RESULTS

**Part I.** Our studies have confirmed the presence of high levels of potassium ions in whole blood stored in ACD solution. The plasma concentration parallels the duration of storage after collection, being of the order of 20 to 25 mEq/L in 3 week old blood. Fresh blood, 3 week old blood, and blood with added potassium (to a concentration of 40 mEq/L) had the potassium concentrations reduced to 1 mEq/L after column passage.

The potassium removing effectiveness of a single column was observed to be at least 10 mEq (the equivalent of 25 mEq/L of potassium in the plasma of 2500 cc. of blood). *The normal erythrocyte survival is in agreement with previous studies.*[9] There was no abnormal erythrocyte fragility.

Sodium concentrations rose from 140 (control) to 148 mEq/L. Calcium concentrations in the plasma of fresh blood fell to 1 mEq/L. The plasma calcium levels of ACD collected blood changed little, apparently indicating the citrate calcium bond. (The physico-chemical status of the small amount of calcium which *was* removed by the column is unknown.)

## METHOD

**Part II.** The freedom from toxic effects of blood collected through an ion exchange column was demonstrated by bleeding and re-transfusion experiments in dogs. Morphine sulfate premedication of 3 mg./kg. of body weight was given. Femoral, carotid, and external jugular arteries and veins were cannulated as necessary using 2 per cent procaine local anesthesia. Systolic blood pressures were read from a mercury manometer. After the experiments the wounds were closed with catgut. The dogs were observed for 4 days to several weeks. Ten dogs were bled and re-transfused in increments of 10 per cent to 60 per cent * to a total of 250 per cent of blood volume. In 15 dogs urethral (Foley) catheters were passed to the junction of the superior vena cava with the right atrium and into the inferior vena cava cephalad of the hepatic veins (see Fig. 2). The balloons were inflated with 5 cc. of water to obstruct venae cavae and azygos inflow, demonstrated by a prompt fall in blood pressure to near zero levels. Repeated bleeding of 10 per cent of blood volume was followed by supra-aortic infusion at 14 mm./Hg pressure after inflation of the catheter balloons produced cardiac inflow occlusion. This was repeated to a total exceeding 250 per cent of the blood volume. All bleeding and re-transfusion was accomplished in 30 to 90 minutes.

* 10 per cent of body weight was used as the weight of the total blood volume.

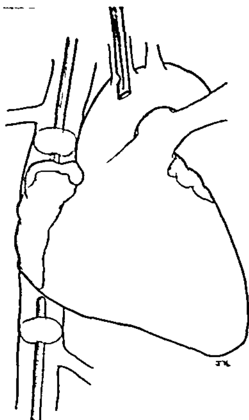


Fig. 2. Balloon catheters permit occlusion of venous return to the heart without thoracotomy.

RESULTS

Part II. All dogs survived. The control clotting times of 3 minutes rose to 7 to 9 minutes after the experiment. The blood pressure returned to normal after each transfusion upon the deflation of the venae cavae balloons. The dogs' serum electrolytes immediately after the experiments averaged potasssium 2.9, sodium 149 and calcium 4.0 mEq./L. One hour post-experiment values were 3.2, 150, and 4.5, and 2 hour concentrations were 3.2, 150 and 5.5 mEq./L. respectively.

DISCUSSION

In 1950 C. W. Walter introduced to the Surgical Forum the sulfonated polystrene copolymer ion exchange column as the basis of a technique for the collection, storage and administration of unadulterated whole blood.[1] We have used that resin column in these experiments. The original communication placed emphasis upon the decalcifying action of the resin column, thereby rendering blood incoagulable without the addition of substances with detrimental physiological actions, notably citrate. Investigators subsequently have employed ion exchange collected blood in experiments but have added magnesium and potassium[5] before transfusing blood in volumes comparable to the large volumes in the current experiments. While the addition of chemicals to reconstitute normal ion concentrations is reasonable since it may preclude some ion deficiency side-effects, this has been shown to be unnecessary for survival. There is a rapid return of plasma electrolyte concentrations toward normal values to the asymptomatic range.

Ivy[4] in 1943, Bruneau and Graham[2] in 1943 and Cookson[3] in 1954 published the results of dog bleeding and retransfusion experiments. All dem

onstrated the marked toxicity of citrated blood contrasted with minimal lethality of heparin The latter introduced prolonged clotting times The experiments reported in the present paper were devised to make similar observations while bleeding greater total volumes in less time using only the ion exchange blood Two distinct advantages of the use of ion exchange blood compared with heparin are the permanent anticoagulation and the minimal derangement of clotting times in the recipient

The preceding statements have referred to unadulterated whole blood (non citrated) It is to be noted that the citrated whole blood passed through the ion exchange column to remove excess potassium must be assumed to retain its qualities of citrate toxicity The cautions of Adams[1] against transfusing citrated blood at a rate exceeding 1 liter per hour must be remembered as well as the observations of others of the hazards of even smaller quantities of citrated blood in patients with liver disease and in cardiac and pediatric patients

It is recognized that not all instances of acute renal failure will develop hyperpotassemia However it is equally certain that the cause of death in renal failure appears to be related to hyperkalemia in many instances Tissue destruction stress hypovolemic shock and the rapid transfusion of large quantities of blood coincide with elevated plasma potassium The rewarming of blood prior to transfusion to aid the movement of potassium from the plasma into the erythrocytes is time consuming and uncertain Glucose insulin testosterone and digitalis therapy are best reserved for the unavoidable endogenous potassium accumulations in the extracellular space To the patient s advantage excessive exogenous plasma potassium may be kept from the blood recipient simply and inexpensively in the transfusion process described herein

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# ETIOLOGY AND PREVENTION OF SLOUGHS PRODUCED BY L-NOR-EPINEPHRINE*

JACQUES Y. BERBEN, MILTON F. BRYANT, AND JOHN M. HOWARD

A distressing and not infrequent complication associated with the administration of a continuous intravenous drip of l-nor-epinephrine has been the production of extensive soft tissue sloughs. In an effort to clarify the etiology of nor-epinephrine sloughs and to determine how these sloughs could be prevented, the following studies have been carried out.

## METHOD

The studies were performed on mongrel dogs using a test solution of 5 per cent glucose in water containing 8 $\mu$g. l-nor-epinephrine/cc.

**Etiology.** At the onset of this study it was recognized that subcutaneous infiltration of nor-epinephrine would produce soft tissue sloughs[1, 2]. Various authors[3, 4, 5] have also suggested that sloughs may result from the intravenous administration of solutions containing nor-epinephrine. The current report deals with the local effects of the drug when administered intravenously or subcutaneously. Studies include observations on: (1) The local effects of the intravenous injection of nor-epinephrine test solution when the solution is administered to normal dogs as follows: a) Rapid injection of 10 cc. of test solution into the vena cava and femoral veins in 5 dogs. b) Rapid injection of 10 cc. of test solution into medium sized veins in 5 dogs. c) Drip injection (50–70 minutes) of 100 cc. of test solution into the vena cava and femoral veins in 5 dogs. d) Continuous intravenous drip injection for periods up to 72 hours into medium sized veins in 5 dogs.

(2) The local effects of l-nor-epinephrine test solution when given intravenously to dogs in hemorrhagic shock and to dogs whose inferior vena cava had been ligated were studied in 6 dogs.

(3) The local effects of the subcutaneous injection of l-nor-epinephrine test solution were studied by: a) Rapid injection of 100 cc. of test solution into the subcutaneous tissues in 12 dogs. b) Subcutaneous drip injection (50–70 minutes) of 100 cc. of test solution in 12 dogs.

**Prevention of sloughs produced by nor-epinephrine.** Once a standard preparation was achieved that would permit the uniform production of a sloughing wound following the subcutaneous drip infusion of nor-epinephrine, prevention of the necrotizing effect was attempted as follows: (1) Infusion subcutaneously of saline, histamine, procaine, hyaluronidase and papaverine in respective experiments following the extravasation of nor-epinephrine in 10 dogs. Effect of perivascular sympathectomy immediately after subcutaneous extravasation of nor-epinephrine in 2 dogs. (2) Effect of Regitine on the subcutaneous extravasation of nor-epinephrine: a) Subcutaneous drip injection (50–70 minutes) of 100 cc. of test solution followed by immediate intravenous injection of 10 mg. of Regitine in 5 dogs. b) Subcutaneous drip injection of 100 cc. of test solution followed by immediate and delayed (1, 2

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* From the Whitehead Department of Surgery, Emory University School of Medicine and Grady Memorial Hospital, Atlanta, Georgia. Supported in part by Department of Army Grant (DA-49-007-MD-732).

3 4 6 8 10 12 16 18 20 and 24 hours) injection of 10 mg of Regitine into the area of nor epinephrine extravasation in 15 dogs

OBSERVATIONS

**Etiology** *Intravenous infusion* The direct local effect associated with the intravenous administration of nor epinephrine test solution into medium sized veins was the production of marked venous spasm of the vein receiving the injection or drip infusion The spasm or contraction lasts from 4 to 6 hours and then disappears even though the drip infusion is continued Spasms of large veins (femoral iliac and vena cava) could not be produced with the nor epinephrine test solution Leakage of the test solution through the vein wall was not observed even though the drip injection was maintained for periods up to 72 hours Studies performed on dogs in hemorrhagic shock and on dogs with their vena cava ligated did not differ from the results obtained in normal dogs

*Subcutaneous infusion* Rapid injection of 50 to 100 cc of test solution into the subcutaneous tissues did not produce soft tissue necrosis however the infusion of the same quantity of test solution into the subcutaneous tissues over a 30–60 minute period routinely produced tissue necrosis Concentration of nor epinephrine was not important as 4 to 8 cc of concentrated nor epinephrine was injected subcutaneously without producing sloughs

**Prevention of sloughs produced by nor epinephrine** Saline histamine procaine hyaluronidase papaverine and sympathectomy were not effective in preventing sloughs produced by extravasation of the nor epinephrine solution Regitine injected into and about the margins of the subcutaneous infiltration of nor-epinephrine test solution routinely prevented the formation of soft tissue sloughs up to periods of 12 hours following extravasation of the test solution (See Fig 1) Prevention of sloughs by Regitine after the lapse of 12 hours was unpredictable however some effect was noticed 18 hours following the infiltration of the nor epinephrine test solution Regitine did not affect the outcome of tissue necrosis if injected 18 or more hours following the extravasation

DISCUSSION

In spite of clinical experience we have found no evidence that the intravenous administration of nor epinephrine will produce soft tissue sloughs Drip infusion of solutions containing nor epinephrine produce marked con



Fig 1 Right thigh—effect of extravascular infiltration of nor epinephrine Left thigh—prevention of tissue necrosis by injecting Regitine into the nor epinephrine extravasation

traction of medium sized veins receiving the infusion This spasm lasts from 1 to 6 hours and then is replaced by venous dilatation even though the infusion is continued This would seem to rule out the possibility that prolonged spasm of veins in the region of a drip infusion of nor epinephrine might cause tissue necrosis as suggested by Kurland *et al*[3] and Uricchio *et al*[4] Humphries and associates[5] postulated that spasm of the vasa vasorum might interfere with the nutrition of the walls of the vein and lead to increased permeability and extravasation We could not demonstrate although we could entirely rule out leakage through the walls of veins receiving nor epinephrine even though the test solution was given continuously for periods up to 72 hours Skin necrosis did not occur in the absence of extravasation

Wall and Hanlon suggested that cutaneous ischemia induced by shock would be aggravated by nor epinephrine and lead to tissue necrosis In the current experiments soft tissue sloughs did not result from the intravenous administration to dogs in hemorrhagic shock

Bergmann[8] suggests that the hydrostatic pressure of the drip infusion of nor adrenaline was much higher than the venous pressure so that the nor adrenaline passed *via* retrograde flow through the tributary veins to capillaries and arterioles Intense spasm of regional capillaries and arterioles then occurred which led to ischemia and gangrene Even with the inferior vena cava ligated so as to force the nor epinephrine solution into the tributary veins soft tissue sloughs could not be produced

The rapid injection of 50 to 100 cc of the test solution into the subcutaneous tissues failed to produce sloughs The same concentration and quantity of nor epinephrine infused into the subcutaneous tissues over a period of 30 to 60 minutes routinely produced soft tissue sloughs Why this occurred is not readily explainable

Regitine (Phentolamine Ciba) is dramatically effective in preventing tissue necrosis by solutions containing nor epinephrine if injected into the extravasated area immediately or up to 12 hours following extravasation Between 12 and 18 hours it was found that the extent of tissue necrosis was definitely diminished Regitine did not affect the outcome of tissue necrosis if injected 18 or more hours following extravasation The action of Regitine appears to be local as Regitine injected intravenously or into the subcutaneous tissues in the leg opposite the extravasation will not prevent tissue necrosis Regitine is so powerful in its action that it presumably prevents the action of nor epinephrine upon the smooth muscle cells Other potent pharmacologic agents (procaine hyaluronidase papaverine and histamine) were not effective in preventing soft tissue sloughs produced by extravasated solutions containing nor epinephrine

SUMMARY

1 Soft tissue sloughs associated with the intravenous administration of solutions containing nor epinephrine are due to extravascular infiltration of the solution

2 Soft tissue sloughs can be prevented by preventing the extravasation of solution containing nor epinephrine

3 If extravasation inadvertently occurs tissue necrosis can be minimized or prevented by diluting 10 mg of Regitine in 20 cc of saline and injecting this solution into and about the margins of the extravasation

2, 4, 6, 8, 10, 12, 16, 18, 20 and 21 hours) injection of 10 mg. of Regitine into the area of nor-epinephrine extravasation in 15 dogs.

OBSERVATIONS

**Etiology.** *Intravenous infusion.* The direct local effect associated with the intravenous administration of nor-epinephrine test solution into medium sized veins was the production of marked venous spasm of the vein receiving the injection or drip infusion. The spasm or contraction lasts from 4 to 6 hours and then disappears even though the drip infusion is continued. Spasms of large veins (femoral, iliac and vena cava) could not be produced with the nor-epinephrine test solution. Leakage of the test solution through the vein wall was not observed even though the drip injection was maintained for periods up to 72 hours. Studies performed on dogs in hemorrhagic shock and on dogs with their vena cava ligated did not differ from the results obtained in normal dogs.

*Subcutaneous infusion.* Rapid injection of 50 to 100 cc. of test solution into the subcutaneous tissues did not produce soft tissue necrosis; however, the infusion of the same quantity of test solution into the subcutaneous tissues over a 30–60 minute period routinely produced tissue necrosis. Concentration of nor-epinephrine was not important as 4 to 8 cc. of concentrated nor-epinephrine was injected subcutaneously without producing sloughs.

**Prevention of sloughs produced by nor-epinephrine.** Saline, histamine, procaine, hyaluronidase, papaverine and sympathectomy were not effective in preventing sloughs produced by extravasation of the nor-epinephrine solution. Regitine injected into and about the margins of the subcutaneous infiltration of nor-epinephrine test solution routinely prevented the formation of soft tissue sloughs up to periods of 12 hours following extravasation of the test solution. (See Fig. 1.) Prevention of sloughs by Regitine after the lapse of 12 hours was unpredictable; however, some effect was noticed 18 hours following the infiltration of the nor-epinephrine test solution. Regitine did not affect the outcome of tissue necrosis if injected 18 or more hours following the extravasation.

DISCUSSION

In spite of clinical experience we have found no evidence that the intravenous administration of nor-epinephrine will produce soft tissue sloughs. Drip infusion of solutions containing nor-epinephrine produce marked con-



Fig. 1. Right thigh—effect of extravascular infiltration of nor-epinephrine. Left thigh—prevention of tissue necrosis by injecting Regitine into the nor-epinephrine extravasation.

3.0 to 4.0 cm. within the pulp of the lower splenic pole. The manometer bases were placed at the level of the branch vein or venostatic level.

In the Group 2 series, a #18 or more preferably a #15 gauge needle was introduced through the left 9th or 10th costal interspaces in the mid or posterior axillary line. With the manometer base at the hub of the needle, pressures were read at splenostatic level. Three observations of the leveling meniscus were recorded and averaged to the nearest centimeter. No calibration was interposed for the mean anatomical level of the portal vein or the right atrium.

Simultaneous recordings at venostatic and splenostatic levels indicated that the former is 10.0 to 12.0 cm. higher than the latter or the anatomical difference in manometer bases. This factor is not interposed but must be considered when interpreting our results. The fluid medium was normal saline with 20 mg. of heparin sodium added to each 100 cc.

The 15 control patients had no evidence of portal venous disease and were usually undergoing gallbladder or gastric surgery. The simultaneous portal and pulp manometrics were taken at venostatic level upon opening the abdomen, with the peritoneal cavity free of surgical instruments.

The 40 patients in Group 2 were subdivided. *Subgroup A* was composed of 32 patients with clinical and laboratory evidence to support the diagnosis of cirrhosis. Twenty-nine had liver biopsies which were reported as follows: Laennac's cirrhosis 21, periportal fibrosis 4, fatty changes 2, biliary stasis and cholangitis 1, and biliary cirrhosis 1. For the purposes of the current study all were considered "cirrhotics." In 6 the livers were nonpalpable.

*Subgroup B* consisted of 6 patients with liver biopsies confirming metastatic disease. Two patients who were placed in a miscellaneous group had diagnoses of "congenital hemolytic jaundice" and "myelogenous leukemia with hypersplenism."

The Group 2 studies were performed in the x-ray department with the patients supine on the Sanchez-Perez Automatic Seriograph which produced 8 exposures at one-second intervals. Local anesthesia was used in all but 6 instances, while the contrast material injected was 30 cc. of Urokon (R) sodium 70 per cent (sodium acetrizoate).

All but 4 patients in *Subgroup A* were esophagoscoped while esophagograms were routinely performed. Two of the 4 unscoped individuals had definite evidence of varices on portography. Splenomegaly was determined by palpation only, though in at least 3 patients it appeared enlarged on scout films. Standard liver function studies were repeatedly performed on all patients.

The *Subgroup A* results were examined with respect to ascites, jaundice, splenomegaly, esophago-gastric varices and gastrointestinal hemorrhage.

The portograms were rated as *normal*, *abnormal* or *unsatisfactory*. In the normal film the splenic vein was of small caliber (1.0 cm. or less in diameter), transverse in position and, with the exception of the portal, the only vein visualized. For an abnormal rating or anatomical evidence of hypertension 2 or more of the following were necessary: a large tortuous splenic vein (1.0 cm. or more in diameter), identifiable coronary, short gastric, mesenteric or perisplenic veins, or varices. Preferential flow through collaterals was considered pathognomonic of elevated tension, and more than an 8-second delay in solid opacification of the liver was considered obstructive

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## SPLENIC PULP MANOMETRICS*

*Preliminary observations of an apparently simple method for measuring portal venous pressure*

FRANCIS C JACKSON AND JOHN L HALLEL

Recently, four new techniques were reported for direct and indirect evaluation of portal venous pressure in the unanesthetized state i e by catheter wedged hepatic vein[1, 2] by cannulation of the portal vein, transhepatically[3] or transabdominally[4] and by the percutaneous needle puncture of the splenic pulp [5, 6]

The current manometric study was suggested by Sherlock and others who demonstrated that the pulp in splenomegalic patients possessed a labile measurable tissue pressure which correlated with wedged hepatic pressures and estimated portal pressures with minimal gradient Splenic pulp pressures and percutaneous portography offered a new combined method of assessing portal circulation

Our study sample consisted of 61 splenic punctures and 31 portagrams performed upon 55 hospitalized patients With the exception of a control group patients with disease of the portal circulation were selected for study

All pressures were recorded on the standard spinal fluid manometer calibrated to 60 0 cm (B&D#5027) The technique of Child[8] was used in the Group I or control series to record simultaneous pulp and portal vein pressure measurements A #19 polyethylene catheter through a major branch was introduced into the portal vein while a #13 gauge needle was placed

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equal or 10 0 cm In 11 studies there was little or no gradient Where a reverse gradient seemed present i e the portal greater than the pulp pressure by more than 1 0 cm in 2 patients we suspected that there were unrecognized technical errors

*Table 1 Splenic Pulp Pressure at Splenostatic Level in Group 2*

| I SUBGROUP A (CIRRHOSIS) | CLINICAL SIGNS | NO PATIENTS EXAMINED | MEAN PULP PRESSURE (Cm N S) | EXTREMES (Cm N S) |
|---|---|---|---|---|
| Mean pressure in 32 patients—33 0 cm | 1 Splenomegaly | 19 | 40 0 | 30 0 — 58 0 |
| | 2 Asplenomegaly | 13 | 25 0 | 11 0 — 31 0 |
| | 1 Varices | 16 | 40 0 | 30 0 — 58 0 |
| | 2 No varices | 16 | 27 0 | 14 0 — 43 0 |
| | 1 G I Hemorrhage | 17 | 35 0 | 16 0 — 52 0 |
| | 2 No G I Hemorrhage | 15 | 32 0 | 14 0 — 58 0 |
| | 1 Hemorrhage from varices | 10 | 40 0 | 31 0 — 52 0 |
| | 2 Hemorrhage from other sources | 7 | 26 0 | 16 0 — 32 0 |
| | 1 Jaundice (serum bilirubin >2.5 mg %) | 11 | 31 0 | 16 0 — 52 0 |
| | 2 No jaundice (serum bilirubin <2.5 mg %) | 21 | 33 0 | 11 0 — 58 0 |
| | 1 Ascites | 8 | 35 0 | 20 0 — 58 0 |
| | 2 Ascites and jaundice | 9 | 37 0 | 31 0 — 52 0 |
| II SUBGROUP B (LIVER METASTASES) Mean pressure in 6 patients—27 0 cm | 1 Hepatomegaly | 5 | 28 0 | 12 0 — 38 0 |
| | 2 Hepatomegaly & splenomegaly | 2 | 32 0 | 25 0 — 38 0 |
| III MISCELLANEOUS | 1 Congenital hemolytic jaundice | 1 | 27 0 | |
| | 2 Myelogenous leukemia | 1 | 0 0 | |

**Group 2.** The results of related clinical data and pulp pressures are tabulated in Table 1

There appeared to be a definite relation between the clinical stigmata of

liver disease Dye in the subcapsular space or poor opacification of the portal system was an unsatisfactory study

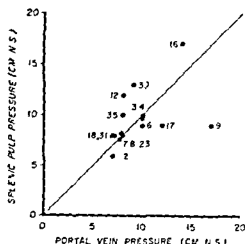



Fig 1 The manometer base levels were located at the level of the cannulated portal branch vein or the "venostatic level" in the abdominal wound In 9 of the 15 studies the pulp pressure was within 1 0 cm of the portal Where a difference of more than 1 0 cm existed as in 7 a pulp gradient was noted in 6 There was little or no gradient in 11 observations

RESULTS

*Group 1* Figure 1 is a plot of the 15 simultaneous uncorrected portal and pulp pressures at venostatic level The means of the 2 pressures were

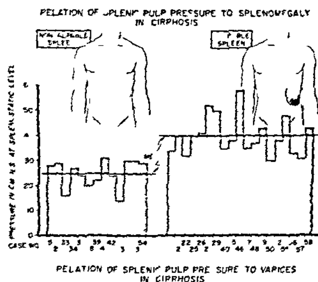



Fig 2 There were no palpable spleens in 10 patients with pulp pressures below 29 0 cm while 19 of 22 spleens were palpable with manometrics of 30 0 cm or over

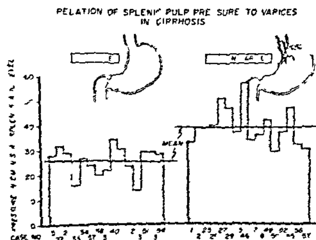



Fig 3 Varices were present in 16 of 22 patients with pulp pressures over 30 0 cm and 8 of 9 over 40 0 cm All with varices had splenomegaly

equal or 10.0 cm. In 11 studies there was little or no gradient. Where a reverse gradient seemed present, i.e., the portal greater than the pulp pressure by more than 1.0 cm in 2 patients, we suspected that there were unrecognized technical errors.

*Table 1. Splenic Pulp Pressure at Splenostatic Level in Group 2*

| I. SUBGROUP A (CIRRHOSIS) | CLINICAL SIGNS | NO. PATIENTS EXAMINED | MEAN PULP PRESSURE (Cm. N.S.) | EXTREMES (Cm. N.S.) |
|---|---|---|---|---|
| Mean pressure in 32 patients—33.0 cm. | 1. Splenomegaly | 19 | 40.0 | 30.0 — 58.0 |
| | 2. Asplenomegaly | 13 | 25.0 | 11.0 — 31.0 |
| | 1. Varices | 16 | 40.0 | 30.0 — 58.0 |
| | 2. No varices | 16 | 27.0 | 14.0 — 43.0 |
| | 1. G.I. Hemorrhage | 17 | 35.0 | 16.0 — 52.0 |
| | 2. No G.I. Hemorrhage | 15 | 32.0 | 14.0 — 58.0 |
| | 1. Hemorrhage from varices | 10 | 40.0 | 34.0 — 52.0 |
| | 2. Hemorrhage from other sources | 7 | 26.0 | 16.0 — 32.0 |
| | 1. Jaundice (serum bilirubin >2.5 mg. %) | 11 | 31.0 | 16.0 — 52.0 |
| | 2. No jaundice (serum bilirubin <2.5 mg. %) | 21 | 33.0 | 11.0 — 58.0 |
| | 1. Ascites | 8 | 35.0 | 20.0 — 58.0 |
| | 2. Ascites and jaundice | 9 | 37.0 | 31.0 — 52.0 |
| II. SUBGROUP B (LIVER METASTASES) Mean pressure in 6 patients—27.0 cm. | 1. Hepatomegaly | 5 | 28.0 | 12.0 — 38.0 |
| | 2. Hepatomegaly & splenomegaly | 2 | 32.0 | 25.0 — 38.0 |
| III. MISCELLANEOUS | 1. Congenital hemolytic jaundice | 1 | 27.0 | |
| | 2. Myelogenous leukemia | 1 | 0.0 | |

**Group 2.** The results of related clinical data and pulp pressures are tabulated in Table 1.

There appeared to be a definite relation between the clinical stigmata of

portal hypertension and the degree of splenic pulp pressure (Fig 2) Splenomegaly apparently develops or is almost always present with pulp pressures over 30 0 cm When the 35 0 cm level is reached both varices and the large spleen are usually demonstrable Varices were present in only 4 of 18 below 35 0 cm (Fig 3)

From these observations there does not appear to be a very definite relationship of pulp pressure to serious parenchymal damage in cirrhosis although when ascites and jaundice were associated with splenomegaly and varices the pulp tension was correspondingly elevated

The mean of 17 patients with hemorrhage was 35 0 However when the bleeding proved to be from varices the mean was 40 0 cm

Apparently it may be impossible to record a valid tissue pressure in a cellular disease which involves the pulp We obtained no pressure in a huge spleen of a patient with myelogenous leukemia

Three patients were studied before and after end to side porta caval shunt Two of the pulp pressures were reduced from pulp tensions of 40 0 and 52 0 cm to 17 0 and 26 0 cm respectively One pressure of 35 0 cm was 38 0 cm postoperatively This caused some concern regarding the patency of the shunt until the portagram demonstrated an incomplete caval compression by the liver The pressure in the inferior vena cava had been 24 0 cm and the narrow gradient across the shunt was a source of concern at surgery

There were 26 portagrams sufficiently satisfactory for evaluation in *Subgroup A* Twenty three were abnormal by our definition The mean pulp pressure was 35 0 cm with extremes of 22 0 and 58 cm Only 5 abnormal portagrams were noted with pressures below 30 0 cm The mean of the 3 normals was 28 0 cm

With metastatic tumor in the liver portagrams usually demonstrated the avascular nodules A tremendous collateralization was produced between the splenic and bifid renal veins where the splenic vein was invaded in 1 patient This pulp pressure was 32 0 cm

DISCUSSION

A free communication between the pulp spaces or sinusoids and the richly anastomotic intrasplenic venules has been given as the principal anatomical relation between the spleen and the portal circulation [2] Its intimate relation with the splanchnic system marks it as an integral part in rather than a dependent organ of the general circulation

While much study remains it would seem that the pulp pressure can be a reliable index of portal pressure Clinical support for this contention would seem to be the relation of this tissue pressure to splenomegaly and varix formation

When the pulp tissue pressure exceeds 35 0 cm of saline at splenostatic level and is associated with collateralization on portagram gastrointestinal hemorrhage could be anticipated

There are definite factors of morbidity in this study which should not be minimized Early in our work it was necessary to remove 2 spleens in Group 1 and 1 in Group 2 We have ascribed these to misguided effort and poor technical judgment In Group 2 slight or no pain was noted during or following the study in 26 patients severe pain in 6 fever in 6 hypotension in 3 fainting and requiring transfusions 1 each

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# PLASMIN LYSIS OF EXPERIMENTAL RADIOACTIVE PULMONARY EMBOLI, WITH CONSIDERATION OF A PLASMIN INHIBITION TEST*


MICHAEL HUME WILLIAM W L GLENN PAUL J ROSENBAUM
PAUL H GUILFOIL AND DANIEL L KLINE


Previous work in this laboratory by Scott† and Glenn in which lysis of experimental clots using plasmin was attempted demonstrated a need for detecting clot lysis as it progressed A method was devised by which the dissolution of a radioactive clot the cells of which were tagged with chromium[51] was studied by sampling the blood for changes in radioactivity during lysis Somewhat similar methods have been reported elsewhere previously[1 -] In our earlier work the radioactive clot was introduced into the arterial circulation and in these experiments considerable fragmentation of the clot occurred Furthermore it was difficult to assess the final result by autopsy because any remaining fragments of clot were scattered into a large capillary bed and were unavailable for evaluation Some of these difficulties were met by introducing the clot into the venous side of the circulation particularly into the inferior vena cava from which it was allowed to pass into the right side of the heart and from there to the pulmonary artery and its branches The slower circulation of the venous side tended to disturb the clot less and examination of the

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†Scott F B Senior thesis in partial fulfillment of requirements for M D degree 1955

major branches of the pulmonary artery at autopsy was relatively easy The present study considers first the loss of substance from a standard radioactive clot after it is introduced into the dog and second the effectiveness of plasmin lysis of these experimental clots Changes in the clotting mechanism are discussed and a plasma inhibition test described

METHODS

**Preparing the standard radioactive clot** Prior to the cannulation of the inferior vena cava blood was withdrawn for the formation of the radioactive clot Fifteen ml was placed into each of 2 bottles containing 4 ml of ACD solution Sodium radiochromate (Rachromate Abbott) was introduced into 1 of these the amount varying from 0 15 to 0 5 mc The blood was incubated for 1 hour at 37°C with occasional gentle rotation of the bottle The plasma was then withdrawn and discarded and the remaining cells were washed 3 times with cold isotonic saline solution to remove unbound sodium radiochromate The tagged cells were resuspended in plasma obtained from the second bottle This blood was introduced into a glass tube which had been rinsed with a strong thrombin solution (Topical Thrombin Upjohn) Clotting quickly occurred and retraction was allowed to proceed at 37°C for 1 hour Loose cells and serum were then removed by washing saline solution past the clot In a second washing technique the clot was placed on a Buchner filter paper and was washed with the aid of suction A third method employed a circulating chamber with connecting tubing that passed through a Sigmamotor pump The simplest and most rapid method proved to be that using the Buchner funnel The clot was weighed after washing and then floated off the filter paper and reintroduced into the glass tube for injection into the inferior vena cava

**Introducing the clot** The dog was anesthetized with nembutal Through a transperitoneal incision the vena cava was cannulated cephalad with a wide diameter glass cannula The glass tube containing the clot was attached to the cannula in the cava and the clot was gently flushed into the vein The cava was ligated when the cannula was removed and the wound closed tightly As the animal was sacrificed at the conclusion of the experiment aseptic technique was not observed Polyethylene cannulae (PE 205, Clay Adams) placed in the femoral artery and right heart (*via* the jugular vein) allowed monitoring of arterial and central venous pressures during embolization

**Preparation and dosage of plasmin** Human plasmin prepared by the method of Kline was used (This preparation contained 1 mg of protein nitrogen/ml of solution ) In most of the experiments 0 5 ml containing 0 5 mg /kg of body weight was given usually as a single dose injected into the catheter that lay in the superior vena cava The injection was given over a one minute period Occasionally a second injection was given The activity of the enzyme was tested against a standard clot for each new batch of plasmin prepared In every instance the enzyme conformed to the standards of activity as described by Kline

**Plasmin inhibition test** The test as performed is generally comparable to the test used by Tillett Johnson and McCarty[3] to measure streptokinase inhibition in patients using clots of their whole blood except that the enzyme used here was plasmin instead of streptokinase Serial halving dilutions of

human plasmin were made in 12 x 75 mm test tubes beginning with full strength plasmin (1 mg/ml) and carried to 9 tubes (1:256 plasmin). Each tube contained 0.1 ml of (diluted) enzyme. Into each 1 ml of dog's blood was placed and mixed by tapping the tube. With practice the procedure could be completed within 4 minutes and before clotting had occurred. The tubes were placed in a 37°C water bath and a note made of the time of clotting and subsequently the time of complete lysis. The end point was taken as the last tube in which complete lysis occurred and was reported as the dilution of enzyme, i.e., 1:2, 1:4, et cetera. The end point was reached in from 20 minutes to 4 hours. It was read at the last tube with complete lysis whether or not any clot remained in a tube with a higher concentration of plasmin.

**Determination of the progress of clot lysis.** After the wound was closed a probe type scintillation counter was positioned over the precordium at the point of maximum radioactivity. This was monitored by a Leeds Northrup recorder *via* a scaler and count rate meter. Two milliliter samples of blood were taken at frequent intervals during the experiment and placed in 10 x 75 mm tubes. Each of these tubes was subsequently counted in a well type scintillation counter. Final estimate of the completeness of lysis was by autopsy with careful dissection of the major branches of the pulmonary artery. In some cases any remaining fragments were collected and weighed.

**Studies of clotting factors.** Coagulation time of the whole blood was determined in 10 x 75 mm tubes by tilting a 2 ml sample at 1 minute intervals until a solid invertible clot occurred. This was carried out at room temperature. A rapid estimation of fibrinogen was carried out in some cases. The method used was essentially that described by Schneider[4] except that in some instances Ringer's solution was replaced as a source of calcium by a Veronal isotonic saline buffer containing 0.0025 M calcium chloride. Paper electrophoresis of plasma in a Spinco Durrum cell was performed in some experiments to demonstrate the presence or absence of fibrinogen. Veronal buffer at pH 8.6, ionic strength 0.1, was used with heparin added to the buffer to prevent clotting. Running time was 16 hours at 7 milliamperes per 8 strips and the strips were then stained with bromphenol blue.*

## RESULTS

**The *in vitro* loss of radioactivity from the standard clot.** Four standard clots were placed for varying periods in a bath of dog serum at 37°C. In 1 instance the serum was circulated through a circulation chamber with a Sigmamotor pump. These 4 clots were reduced to about 30 per cent of their starting weight and the serum in which they lay gained radioactivity. Hemolysis was negligible, the increased radioactivity of the wash serum being present as detached tagged erythrocytes. Most of this change in clot weight occurred in the first 6 hours. The clot was about the same length and shape but was noticeably thinner. It did not tend to fragment. Counts per gram of dry clot weight determined by desiccating each clot was about equal for all samples. Water accounted for 70 per cent of the mass of clot weighed after 6 hours or more and 72 per cent of the mass of clot which was desiccated immediately after initial washing.

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*Table 1 Results of plasmin lysis of experimental radioactive pulmonary emboli including autopsy evaluation, changes in radioactivity, plasmin dosage and changes in the clotting mechanism*

| EXPER | CLOT LYSIS AT AUTOPSY | CHANGE OF RADIOACTIVITY (COUNTS PER MINUTE IN THOUSANDS) PRECORDIUM | BLOOD | DOSE OF PLASMIN (MG/KG) | WOUND OOZE | DURATION INCOAG OF BLOOD (HR) | CHANGE IN FIBRINOGEN TITER |
|---|---|---|---|---|---|---|---|
| I I 39 | complete | 11 to 12 | 20 to 22 | 0 7 (given 3×) | slight | 6 | 1 50 to ō |
| I F 38 | complete | 10.5 to 7 7 | 11.5 to 12 4 | 0 7 | none | 3 | 1 200 to ō |
| I E 27 | equivocal | 25 to 23 | 9 to 10 | 0 8 (given 2×) | none | never | 1 100 to 1 10 |
| I E 22 | about half | 25 to 15 | 9 to 15 | 0 5 | yes | 3 | 1 400 to ō |
| PE 21 | more than half | 32 to 18 | 8 to 18 | 0.5 | yes | 4 | 1 100 to ō |
| PE 19 | more than half | 29 to 18 | 5 to 14 | 0 5 | yes | 4 | 1 50 to ō |
| PE 17 | less than half | 31 to 27 | 15 to 19 | 0 5 | yes | 4 | |
| PI 16 | complete | 21 to 12 | 4 to 2 | 0 5 | none | never | |
| I F 15 | about half | 67 to 52 | 33 to 51 | 0 5 (given 2×) | none | never | |
| I I 14 | complete | | 26 to 29 | 0 6 | yes | 8 | returning at 8 hr |
| I I 13 | complete | 1 8 to 2 8 | 0 7 to 3 3 | 0 6 | yes | 1 | |
| I I 12 | three quarters | 64 to 32 | 2 to 11 | 0 5 | | | |
| I I 11 | three quarters | 41 to 24 | 7 to 15 | 0 5 | | 3 | |
| I F 10 | equivocal | 8 to 5 | [illegible] to 5 | 0 5 | | | |
| I F 9 | more than ¾ | 14 to 4 | 3 to 26 | 0 5 | | 4 | |
| I I 8 | more than ¾ | 1 1 to 1 3 | 1 2 to 5 | 1 L SK* | | 3 | |

*Plasminogen 4 mg and streptokinase 4 mg/kg

**Hemodynamic changes due to embolization** Venous and arterial pressure tracings taken during introduction of the clot into the inferior vena cava showed changes suggestive of pulmonary embolization in some experiments In these cases the femoral artery pressure fell and the central venous pressure rose sharply These changes usually returned to previous levels in 2 to 4 minutes In only 2 of 21 experiments using the standard clot did the dog expire as a result of acute pulmonary embolization

**Lysis using plasmin** The results of plasmin lysis are presented in Table 1 Of the 16 experiments complete lysis was achieved in 5 and three quarters lysis or more in an additional 4 Five cases showed lysis of about half of the clot In 2 experiments there was little or no evidence of clot lysis

**Changes in the coagulability of the blood** In most experiments in which clot lysis occurred the blood became incoagulable immediately after the enzyme was given and shortly thereafter the wound began to ooze Fibrinogen levels where determined fell to zero as measured by the rapid assay method and paper electrophoresis showed the fibrinogen band to be absent With the blood incoagulable 0 5 ml of blood mixed with 1 0 ml of blood from a normal animal gave a solid clot which might undergo partial lysis after several hours Table 1 indicates the period during which the blood was incoagulable presence of wound ooze and the results of fibrinogen determinations when these were done

**Plasmin inhibition tests** Seventeen dogs have been used in tests to demonstrate inhibition of human plasmin activity in clots of their whole blood Complete lysis to the fifth tube (plasmin 1 16) was the maximum fibrinolytic effect (the least inhibition) No lysis or lysis in the first tube only (maximum inhibition) occurred in 5 dogs Lysis to the fourth or fifth tube (1 8 or 1 16) was recorded in 7 animals Results varied from day to day for the same animal by 1 or 2 tubes and were uniformly very low (maximum inhibition) under anesthesia

DISCUSSION

The method for preparing and introducing a standard clot has proved to be useful and reproducible Certain reservations exist in view of the fact that the clot is rapidly reduced to about 30 per cent of starting weight by washing in serum It may be supposed that this effect is more or less a constant factor between experiments and we have shown that for a fresh clot a stable state is reached within 6 hours after which changes in radioactivity in the blood and over the precordium may be considered to be due to the action of administered plasmin Several reasons may be suggested for the varying results in clot lysis with plasmin The plasmin preparation contains an activator of the dog s own plasminogen Variations in the extent of this activation may play a part The dosage or the method of administration or variation in the individual s susceptibility to the enzyme could also be responsible The enzyme used has been uniformly active *in vitro* The dose used 0 5 ml /kg probably represents a minimum effective level and was selected so as to disturb the clotting mechanism as little as possible Incoagulability of the blood did usually appear however and in many instances the sutured wound would ooze during the maximum effect of the enzyme It may prove that other methods of administration such as giving the enzyme as a slow infusion may result in better lysis with less clotting alterations How great is the factor played by the individual s inhibitors of plasmin requires

*further study Although the plasmin inhibition test gives some promise of quantitating this aspect of the problem, considerably more experience with it is necessary before its usefulness is established If it can be shown to represent accurately the individual's susceptibility to plasmin under a given set of circumstances it might be effectively applied to indicate the optimum dose required for clot lysis under the conditions present at the moment in question*

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# DIURESIS IN HEMORRHAGIC HYPOTENSION*

EDWIN D SAVLOV AND WILLIAM REGELSON

*The effect of acute hemorrhagic hypotension on renal function in dogs has been studied by Corcoran and Page*[1] *Selkurt*[2] *and Phillips et al*[3] *Although direct measurement of blood flow from the renal vein during hypotension reveals a continuing, significant flow oliguria or anuria supervenes at pressures between 60 and 100 mm Hg*[2] *The exact mechanism of urine flow suppression is not clear Compensatory renal vasoconstriction is present in varying degrees and would certainly seem to have some relationship to the anuria However denervation of the kidney or administration of the adrenolytic 2 piperidinomethyl 1 4 benzodioxane (F933) has not increased urine flow during hypotension*[2]

One of us (W R) has observed an anuric patient without detectable peripheral blood pressure and unresponsive to norepinephrine who diuresed after administration of chlorpromazine Recently Fournel[4] in reporting on the use of chlorpromazine in the treatment of shock, mentioned that dogs bled to levels of 50 mm Hg excreted increased amounts of urine after treatment with the drug The present report is an attempt to confirm these observations in dogs made hypotensive by hemorrhage

METHOD

Eleven mongrel dogs weighing between 5 5 and 16 1 kg were subjected to hemorrhagic hypotension by the method standardized by Wiggers[5] After induction of anesthesia using intravenous sodium pentobarbital 30 mg /kg a polyethylene tube was threaded into the thoracic aorta by way of the

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femoral artery A 3 way stopcock connected the tube with a mercury manometer or a Statham transducer for recording of blood pressure Withdrawal of blood was accomplished through the 3 way stopcock with a 50 cc syringe During each experiment an intravenous infusion of 5 per cent dextrose in water was maintained at 3 cc per minute

Blood was withdrawn rapidly until the blood pressure reached 50 mm Hg and the pressure was maintained for 90 minutes at that level Further bleeding to 30 mm was then done and the pressure sustained at this level for 45 minutes The filtered blood was then reinfused by vein

Urine was evacuated with an indwelling catheter in the bladder which was aspirated and irrigated with air every 15 minutes Gentle supra pubic pressure was also used

All animals were given 300 000 units of procaine penicillin at the beginning of the experiment

Chlorpromazine * 0 2 mg /kg was given intravenously to 7 dogs after the blood pressure had been 50 mm for 45 minutes Four of these dogs received a second similar dose of chlorpromazine after the blood pressure had been dropped to 30 mm Hg

## RESULTS

In the 4 dogs to which no drug was given there was suppression of urine flow at the hypotensive levels (Fig 1) Although complete anuria did not always occur urine output was never more than 0 1 to 0 2 cc per minute

After administration of chlorpromazine at 50 mm Hg there was an almost immediate increase in urine flow in 4 dogs with subsequent suppression on bleeding to 30 mm Hg (Fig 2) Urine flow rate at 50 mm was from 0 6 to 1 2 cc per minute

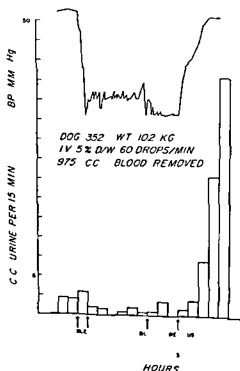



Fig 1 Oliguria at hypotensive levels in a typical control animal

* Supplied by Smith Kline and French Laboratories

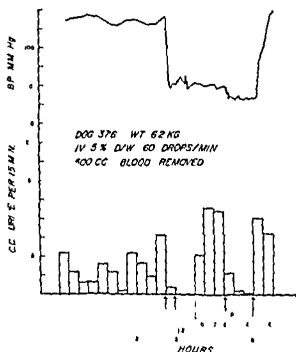



Fig. 2. Increased urine flow at 50 mm Hg following administration of chlorpromazine 0.2 mg per kg. Further bleeding to 30 mm Hg caused recurrence of oliguria.

In 1 of these 4 dogs a second dose of chlorpromazine when the blood pressure had been 30 mm for 15 minutes with extreme oliguria resulted in a second period of diuresis (Fig. 3). Flow at 30 mm was 1.8 cc per minute during the last collection period.

Two dogs showed no increase in urine flow after administration of the drug either at 50 mm or at 30 mm.

The remaining animal is of interest in that no immediate effect was noted after chlorpromazine was given. However, an additional 15 minute period at 18–50 mm Hg was added to the experiment and during this time the dog excreted up to 1.3 cc per minute.

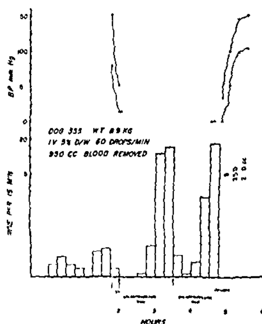



Fig. 3. Increase in urine flow at 50 mm and at 30 mm Hg following successive doses of chlorpromazine 0.2 mg/kg.

Urine excreted during the hypotensive periods contained no protein spe cific gravity was 1 020 to 1 022 Sodium content was 3 7 mEq/L in 1 dog and was 29 0 mEq/L in another during diuresis at 50 mm

DISCUSSION

A number of possibilities may be proposed to explain the increased urine flow in the described circumstances

1 **Effect on posterior pituitary** The secretion of antidiuretic hormone by the posterior pituitary in response to the stimulus of hemorrhage[6] may have been blocked by chlorpromazine Parrish and Levine[7] recently reported a decrease in antidiuretic like substance in the urine of human beings fol lowing the drug

2 **Effect on kidney** a) *On the renal cells* Some direct effect of the drug on the nephron may have either inhibited active water reabsorption in the distal tubules and collecting ducts or prevented proximal reabsorption of solutes The concept of active water secretion in the renal tubules has been postulated in some quarters as a mechanism for urine dilution[8] From this premise it may well be asked if chlorpromazine might have promoted tubu lar water secretion b) *On the blood vessels* An increase in glomerular capil lary permeability allowing indiscriminate passage of large molecules into Bowman s capsule might result in increased fluid passage into the tubules due to lowering of the intravascular colloid osmotic pressure This ultra filtrate would no longer be protein free The absence of proteinuria during the diuresis would seem to make this possibility less likely in the present situation

By its known adrenolytic effects[9] chlorpromazine may have inhibited renal vasoconstriction and lowered renal arteriolar resistance Handley and Moyer[10] injected dibenzyline directly into one renal artery as an adrenergic blockade and demonstrated less depression of renal function in the treated kidney than in the opposite kidney during hemorrhagic hypotension in 2 of 3 dogs Direct renal blood flow measurements in dogs by Brandfonbrener and Geller[11] revealed an increase in flow during hypotension following ad ministration of dibenamine Alteration of renal hemodynamics by chlorpro mazine in a similar manner could be a likely explanation for the present phenomenon and offers an avenue for further experimental exploration

The fact that 2 of the 7 treated dogs did not diurese deserves further com ment It may first be pointed out that under the experimental conditions satisfactory urine output was not uniformly obtained during the preliminary normotensive periods In addition it should be explained that even under conditions of maximal preliminary water diuresis the antidiuretic response stimulated by hemorrhage alone would probably mask minor degrees of diuresis induced by one or a combination of the aforementioned possible mechanisms Other variables include the antidiuretic effect of pentobarbital and variations in the depth of anesthesia

Another factor affecting the consistency of the results is the severe hypo proteinemia observed in all of the dogs The extensive bleeding up to 106 cc /kg plus the dilution of remaining blood by the continuous infusion of glucose solution combined to produce plasma protein levels of 2 5 to 3 0 gm per cent This reduction of plasma colloid osmotic pressure could be a fac tor in maintaining effective capillary filtration pressure If one assumes that

renal arteriolar capillary pressure is approximately ⅔ of aortic blood pressure and that plasma colloid osmotic pressure is roughly proportional to the protein concentration then the capillary filtration pressure will be 10 mm Hg when the blood pressure is 30 mm and the serum protein is 2 5 gm per cent These assumptions disregard factors of arteriolar resistance and tissue pressure but serve to illustrate a means whereby variations of plasma protein may influence urine flow at hypotensive levels It has been well demonstrated by Bojeson[1] in normotensive dogs that a minor depression in plasma protein without change in plasma volume can bring about a dilution diuresis

Subsequent studies to be reported in detail indicate that these factors were instrumental in producing variation It is of interest that although the control animals became equally as hypoproteinemic diuresis did not ensue The present data give no inkling as to the exact role of chlorpromazine in producing diuresis but would certainly seem to confirm Fournel s impression

The dose of chlorpromazine used in this experiment was considerably smaller than that usually given in studies of survival after shock in rats and dogs We gave 2 0 mg /kg to a dog at 50 mm Hg with immediate bradycardia profound drop in blood pressure respiratory arrest and death in a matter of minutes Survival studies using smaller doses of chlorpromazine would be of interest

## SUMMARY

Urine flow up to 1 3 cc per minute was observed in dogs bled to levels of 50 and 30 mm Hg aortic blood pressure This occurred only after administration of a small dose of chlorpromazine

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# THE EXPERIMENTAL PRODUCTION OF A REVERSIBLE STATE OF CHRONIC PROGRESSIVE EDEMA*

JULIUS H JACOBSON II JOHN LARACH AND FERDINAND F McALLISTER

The study of the pathogenesis of the various states of chronic edema has been retarded by the lack of suitable experimental preparations McKee Schilling Tischkoff and Hyatt[1] have contributed to this problem by showing that ascites forms in the dog when the inferior thoracic vena cava is constricted Such preparations however have been somewhat unpredictable regarding the duration of the ascites Some animals have formed ascites slowly while others have died from over constriction The method is not applicable when one wishes to use the dog as its own control after the ascites has been produced or where operation and anesthesia are undesirable in the experimental design

In working out a method for the experimental constriction of larger blood vessels[2] with a pneumatic cuff it occurred to us that this technique might be used to produce easily controllable ascites

## METHOD

A pneumatic rubber cuff† is used to constrict the inferior thoracic vena cava of the dog The cuff with its method of application is illustrated in Figures 1 and 2 It encircles the vena cava and is sewed together by means of

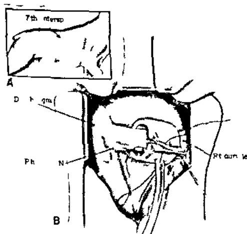



Fig 1

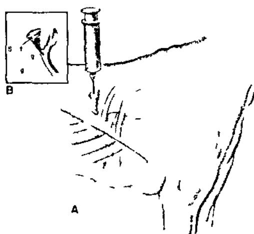



Fig 2 (A) Phantom drawing showing pneumatic cuff in place with needle being introduced subcutaneously into catheter tip (B) Cross section of catheter tip

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With the technical assistance of Mr Gerard M D Alessio
†Made for us by the Davol Rubber Co Providence R I

cloth tapes at either extremity of the balloon Leading from the balloon is a rubber catheter that has at its end a self sealing rubber diaphragm This is led out of the chest and allowed to remain in the subcutaneous tissues after closure of the incision Subsequently, when the animal has recovered from the operative procedure it is possible by injecting water into the self sealing rubber diaphragm to produce degrees of atraumatic sustained occlusion of the vessel The obstruction may then be released in an equally atraumatic fashion by withdrawing the previously injected fluid

There are a number of important steps that must be observed when using the cuff It is essential to employ a cuff approximately twice the diameter of the vena cava A cuff measuring 2 cm in diameter is satisfactory and provides enough leeway to prevent angulation of the vessel The loose areolar tissue surrounding the vena cava should be dissected away for a distance *only* sufficiently large to pass the cuff around the vena cava The optimum site for placement is immediately adjacent to the right auricle rather than near the diaphragm where constant movement is likely to compromise the vena cava The catheter leading from the cuff should be led out through the thoracotomy wound and tunneled for a short distance under the serratus and pectoralis muscles This prevents development of any pneumothorax that might occur around the catheter from an air leak in the skin closure The funnel shaped self sealing rubber diaphragm should be embedded beneath the level of the platysma muscle so as to prevent ulceration of the overlying skin The catheter tip is best left in the non dependent position near the vertebral column so that the seroma that frequently forms in the first post operative week is at a distance from it Such placement helps prevent infection at the time of seroma aspiration and decreases the duration of seroma persistence

The animal is allowed to recover from the cuff installation Control studies are obtained without the clouding influence of the operative procedure, and then the inferior vena cava is constricted Fluid is injected into the catheter tip to the point where the animal becomes unconscious presumably due to a sudden decrease in cardiac output Immediately a small amount of fluid is withdrawn i e 0 25 to 0 5 cc The animal promptly returns to consciousness The resultant degree of constriction is the maximum that is tolerable and ascitic fluid forms within 1 to 2 weeks Attempts to regulate constriction at the operating table or by measurements of femoral venous pressure have been erratic and occasionally lethal

## RESULTS

Ascites has been produced in 9 dogs using the above method In 5 of these a reversible preparation was desired 4 were successful In 3 of the 4 ascites was made to develop and regress twice in the same animal

In Figure 3 pertinent metabolic data from a typical experiment are shown The analytic methods have been previously reported[3, 4] This dog on metabolic balance received a constant intake of 155 mEq of sodium daily and distilled water *ad lib* Intake and output were recorded daily The dog was weighed frequently in the fasting state

It is interesting that upon partial occlusion of the thoracic vena cava sodium retention began immediately and the excretion of sodium was drastically reduced within the first 24 hours Thereafter sodium excretion was

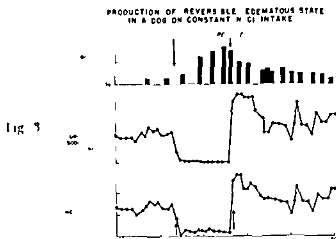



Fig 3

minimal to negligible—usually less than 2 5 mEq /day Chloride excretion followed a similar pattern This dog gained 12 5 kg in the 13 days in which the vena cava was occluded He became markedly ascitic with edema noted in the hind legs Upon release of the constriction diuresis of large amounts of edema fluid began immediately In the ensuing 20 days the animal gradually returned to the control state

SUMMARY

Utilizing a previously inserted pneumatic cuff placed around the inferior thoracic vena cava ascites which is reversible has been produced atraumatically Occlusion of the thoracic vena cava produces almost immediate sustained renal retention of sodium a state which is perhaps analogous to a Chiari syndrome Release of the obstruction produces prompt diuresis with immediate return of sodium excretion The technique is being employed to study renal and hormonal factors in the pathogenesis of ascites Studies relating to the role of the posterior pituitary in formation of ascites are being reported separately in this volume

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# *Pre and Postoperative Problems in Water and Electrolyte Balance and in Nutrition*

## ELECTROLYTE BALANCES IN NEWBORN INFANTS FOLLOWING MAJOR SURGICAL PROCEDURES*

ELEANOR COLLE AND ELSA PAULSEN

A relationship between the metabolic changes following surgery and the release of adrenocortical hormones has been postulated by many investigators. Others have suggested that the adrenal gland plays a permissive role and that renal factors may be prominent in initiating the salt and water retention seen after operation.

Although the adrenal gland of the newborn infant is relatively larger than that of the adult, the major portion of it is composed of so-called fetal cortical tissue which begins to undergo atrophy immediately after birth, leaving the infant with a small amount of adult cortical tissue composed of the zona glomerulosa and the zona fasciculata.[1] This anatomical event, when correlated with such clinical and laboratory observations as the poor febrile response and failure of leukocytosis in infections, low blood sugar levels,[2] high absolute eosinophil counts,[3,4] absence of diurnal eosinophil variation[5] and low levels of 17 hydroxycorticosteroids in the blood[6] has suggested that the neonate is suffering from a relative hypoadrenalism during the first days or weeks of life. Renal immaturity during this same period has been repeatedly demonstrated, tubular function being relatively less efficient than glomerular function.[7]

### METHOD

1. Daily balances for sodium, potassium, chloride and nitrogen were performed on normal newborns and on newborns undergoing surgery. Adequacy of urine collections was insured by the use of indwelling catheters.

2. Blood chemistries were performed on capillary blood: a) sodium and potassium using the Beckman DU flame photometer; b) chloride by titration with mercuric nitrate using diphenylcarbazone as indicator; c) blood urea nitrogen using a micro modification of the Conway cup diffusion method.

3. Chemical analysis of urine and gastrointestinal tract losses were measured using the above techniques. Total nitrogens were measured by micro Kjeldahl. Osmolar concentrations of the urine were determined using the Fiske osmometer.

### RESULTS

**Normal newborns.** Normal full term infants were given 5 per cent glucose or a multiple electrolyte solution (composition: sodium 25 mEq/L, chlo

* Department of Pediatrics, University of Minnesota, Minneapolis, Minn. This work was supported by U.S.P.H.S. Grant No. H 2066.

ride 22 mEq/L and potassium 20 mEq/L) in amounts similar to those administered in the post operative period

While on glucose solution all the infants were in negative balance for sodium potassium chloride and nitrogen The urinary excretions of electrolytes were remarkably constant averaging 1 to 5 mEq/kg/d These figures are in agreement with published values in a similar study by Smith [11]

When electrolyte solutions were offered balances immediately became positive and remained so throughout the period of study The total electrolyte excretion remained about the same (1–5 mEq/kg/d)

Urine concentrations were highest during the period of limited water intake and fell when more water was provided The highest concentration found in this period was 650 mOsm/L the usual range was 150 to 400 mOsm/L

**Newborns following surgery** All infants undergoing surgery in the first week of life were followed on balance studies In some cases it was possible to obtain collections for a pre operative period The majority of the children were operated upon almost immediately following entry to the hospital and the data from these cases have been compared to that from the normal newborns

The total number of infants thus studied is 16 Of these 8 had operations for the relief of gastrointestinal obstruction 5 for repair of a tracheo esophageal fistula and 3 for repairs of sacral myelomeningoceles

Anesthesia was cyclopropane or pentothal in all cases except for 2 of the neurosurgical procedures which were done under local anesthesia

Operating times ranged from 15 min to 3 hr

The group as a whole reveals certain constant features which were never observed in the normal newborn In addition in these respects the post operative period in the newborn differs in several essential features from the post surgical period in the adult

Three such studies are presented in detail as characteristic of the group as a whole

Baby R H was born on 4/28 and admitted on 4/30 Weight at the time of admission was 3 kg She was operated upon on the fourth day of life for repair of a tracheo esophageal fistula Operating time was 3 hr and 25 min Anesthesia was cyclopropane During the 2 days prior to operation the child was in positive sodium and potassium balance Urine output was 65 cc daily (within the range normally found in infants at this age) and the urine concentration was 350 mOsm/L on both days Following surgery the child was in negative balance for sodium potassium and chloride and nitrogen losses increased Negative balances were maintained for 6 days following surgery In addition the absolute amount of electrolytes excreted was greater than before surgery despite a lowered intake Following surgery the urinary output increased and the water balance decreased Associated with this was a decrease in urine concentration to a range of 200–250 mOsm/L which persisted throughout the period of study During the post surgical period the serum sodium was 144 to 152 mEq/L

Baby Cribb was admitted at the age of 54 hr and operated upon almost immediately At surgery a massive meconium peritonitis was encountered An ileostomy was performed Operating time was 3 hr anesthesia was cyclopropane and pentothal No foreperiod collections were available Following

surgery there was a tremendous output of sodium potassium and chloride Nitrogen losses were moderately increased These markedly negative electrolyte balances persisted to the time of the infant s death on the fourth post operative day The urine output was high for an infant of this age and the urine output was greater than fluid intake The urine solute concentration was never higher than 250 mOsm/I The serum sodium concentrations were in the range of 115 to 150 mEq/L throughout the period of study

Baby D L was operated upon at 21 hr of age for low intestinal obstruction Operating time was 2 hr and 25 min and anesthesia was pentothal She weighed 3600 gm at the time of admission This infant not only excreted large amounts of salt in the urine, but did so in spite of large extra renal losses incurred because of intestinal intubation Her urine volume was high and the solute concentration following surgery was never greater than 270 mOsm/L Her serum sodium values post surgically ranged from 140 to 160 mEq/L

These cases represent the type of response seen In every case there was negative balances for sodium potassium chloride and nitrogen in the post operative period The losses of electrolytes were absolutely from 3 to 10 times that seen in normal infants observed during this same period In addition urine volumes increased following surgery and the ratio of urine water to intake water was greater than in the newborn controls The osmolar concentration of the urine remained low during this period The magnitude of these changes correlated roughly with the nature of the surgical procedure the length and type of anesthesia The serum electrolyte concentrations were usually normal or elevated

## DISCUSSION

The post operative period in the adult has been characterized by a period of negative potassium and nitrogen balance a variable degree of sodium and water retention and a variable fall in the serum sodium concentrations [8, 9] *These changes occur during the period when excretion of 17 OH in the* urine is increased although it has not always been possible to correlate the rise in compound F in the blood with the period of salt retention [10]

The newborn infant subjected to surgery responds with an increase in potassium and nitrogen losses similar to that seen in the adult However urinary losses of sodium have been found to increase in those cases in which it was possible to compare them with a pre surgical specimen and to be greatly in excess of those seen in normal newborns on a comparable fluid and electrolyte intake In addition urine output is regularly increased and urinary concentrations are low The serum electrolytes are either normal or (more often) increased

It is known that the infant has diminished renal function in the early days of life However normal infants will concentrate to 600 mOsm/L[11] and the majority of our normal infants have shown concentrations as high as 400 mOsm/L The infant following surgery seems unable to concentrate much higher than 200 to 250 mOsm/L—and this inability to concentrate may remain for many days despite the fact that the child is actually in negative water balance It would seem that the water diuresis seen in the infant may well be the direct result of impairment of kidney tubular function following surgery

Whether the increased electrolyte excretion observed following surgery is a consequence of the primary defect in water metabolism cannot be determined from the data at hand. The role of the adrenal in response to stress has been emphasized by many, although there are others who hold that the adrenal has only a permissive function. If the sodium retention seen in the adult is in some way correlated with an increased secretion of adrenocortical hormones, the following explanations for the failure of the infants to show this response may be offered: (1) The neonate suffers from hypoadrenalism. The evidence of Klein documenting a rise in plasma compound F levels in response to exogenous ACTH and to surgical stress would mitigate this possibility.[12] (2) The immature renal tubule is incapable of responding to a salt retaining hormone. (3) The newborn adrenal produces a salt losing hormone in response to ACTH. Klein has reported the presence of such hormone in young infants after stimulation with exogenous ACTH.[13] (4) The changes seen are due to a pre-existing hormonal imbalance present in the newborn. Antagonism between DOC and other adrenocortical hormones have been reported in patients with Cushing's disease[14] and in patients with adrenal virilism.[15] The newborn infant "pre-treated" during the last trimester with high "doses" of maternal corticoids may react to the normal adrenal secretions called forth by surgical stress in an unexpected manner due to a withdrawal state.

## SUMMARY


1. Balance studies on newborn infants and on newborns undergoing surgery are presented.
2. The infants subjected to surgery are in negative potassium and nitrogen balance during the post-operative period in a manner similar to that seen in adults.
3. The infant post-surgically tends to lose sodium and to have a water diuresis associated with low solute concentrations during this period in contrast to the salt and water retention seen in the adult.
4. Concentrations of electrolytes in the blood are normal or increased during this period.


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# OSMOTIC DIURESIS AS TREATMENT IN SEVERE HYPONATREMIA*

WILLIAM G HAMMOND RODMAN C CARTER JOHN M DAVIS AND FRANCIS D MOORE

With the expanding scope of modern surgery has come an increase in the frequency with which major operative procedures are performed upon patients suffering from chronic depleting illnesses These patients manifesting preoperatively varying degrees of fluid and electrolyte imbalance are often prone to develop severe hyponatremia after operation and are also more sensitive to the consequences of such hyponatremia Severe liver disease and chronic congestive heart failure are the most frequent systemic disorders associated with lowered serum sodium concentrations postoperatively although the chronic semistarvation which accompanies certain lesions requiring operative therapy is of equal significance in this regard

The primary genesis of postoperative hyponatremia in such patients is water loading not salt loss as an increased impetus to renal sodium conservation is a common feature of this syndrome Impaired excretion of water loads occurs during the early postoperative period presumably as the result of increased antidiuretic hormone secretion by the neurohypophysis After the second or third postoperative day the occurrence of continued water retention is most probably related to inadequate solute output as the low urine volumes then are characterized by solute concentrations well below the levels produced by the earlier ADH activity A less negative nitrogen balance and continuing sodium retention now deprive the urine of its two primary solute components—urea and sodium plus accompanying anions Under these circumstances a cumulative positive water balance of several liters may develop insidiously at the rate of only 300 to 400 ml /d The result is marked hyponatremia with the attendant clinical manifestations as described so well by Wynn[1] and others[2]

Low serum sodium concentrations may be restored to normal either by the

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*From the Department of Surgery at the Peter Bent Brigham Hospital Harvard Medical School Boston Massachusetts This work was supported in part by the Atomic Energy Commission and by the Subcommittee on Metabolism in Trauma Advisory Committee on Metabolism Office of the Surgeon General Department of the Army The assistance of Winthrop Laboratories Inc and the Upjohn Company is gratefully acknowledged

addition of sodium (as hypertonic saline infusion) or by decreasing body water Since patients in whom severe post operative hyponatremia most frequently occurs are usually lacking in cardiopulmonary reserve hypertonic saline is contra indicated because further extracellular fluid expansion may precipitate severe pulmonary edema Decreasing body water by marked restriction of water intake may produce elevations of the serum sodium concentration however the rise occurs too slowly to be therapeutically useful A prompt decrease in body water resulting thereby in elevation of serum sodium concentration would be achieved if marked increases in urine flow could be produced by a diuretic agent which did not effect a saluresis These preliminary studies were undertaken to ascertain if mannitol a substance commonly used in renal physiology to promote osmotic diuresis might be such an agent Studies of the effect of mercurial diuretics in similar conditions are included for comparative purposes

## METHOD

Eight studies were performed on 5 patients 3 of whom developed hyponatremia post-operatively and 2 in whom diuretic therapy was being carried out during evaluation for cardiac valvular surgery One patient in this latter group was eunatremic but had not responded to other diuretic measures Hypertonic saline therapy was considered inadvisable in all instances of hyponatremia

Twenty five per cent solutions of mannitol* were administered by intravenous infusion at rates not exceeding 150 ml /hr Mercurials were given subcutaneously in customary dosage

Urine specimens were obtained by indwelling catheter in 4 cases and by voiding in 1 case

Sodium and potassium concentrations in serum and urine were determined by flame photometry Osmolality of serum and urine was measured with a freezing point depression osmometer

## RESULTS

In 4 of 6 instances a significant rise in serum Na concentration occurred following mannitol induced diuresis (See Table 1) However in the other 2 cases oral fluid intake continued to exceed water loss so that the overall fluid balance was unaltered by the mannitol diuresis Two cases having mercurial diuresis show either a drop or no change in serum Na concentration

Of interest also is the fall in serum K occurring in those cases showing elevations of serum Na

Although comparable increases in urine flow resulted from both osmotic and mercurial diuresis the contrasting changes in sodium excretion are striking (Table 2) While mannitol did indeed produce some increase in sodium excretion in 5 of 6 instances this higher rate of sodium loss is still inconsequential with respect to any effect on serum Na concentration or body sodium content

## DISCUSSION

These data show that in the presence of a marked tendency to urinary Na conservation the administration of a nonelectrolyte osmotic diuretic will in

* Merck Sharpe and Dohme supplied the mannitol used

*Table 1 Effect of diuresis on serum osmolality and electrolytes*

| PATIENT | BEFORE DIURESIS | | | AFTER DIURESIS | | |
|---|---|---|---|---|---|---|
| DIURETIC AGENT | SERUM NA mEq/L | SERUM K mEq/L | SERUM OSMOLALITY mOsm/L | SERUM NA mEq/L | SERUM K mEq/L | SERUM OSMOLALITY mOsm/L |
| S E Mannitol | 125 | 4.2 | 260 | 129 | 4.4 | 264 |
| A L I Mannitol | 117 | 6.4 | 250 | 124 | 5.4 | 260 |
| A L II Mannitol | 125 | 4.8 | 261 | 130 | 4.5 | 267 |
| B S Mannitol | 118 | 4.1 | 278 | 138 | 3.3 | 332 |
| W R Mannitol | 134 | 5.1 | 278 | 135 | 5.4 | 280 |
| K O Mannitol | 123 | 5.0 | 266 | 118 | 6.0 | 273 |
| S E Hg | 130 | 4.8 | 265 | 126 | 3.7 | 261 |
| W R Hg | 134 | 4.7 | 278 | 135 | 4.5 | 269 |

crease urine output without significantly altering sodium homeostasis Under clinically appropriate circumstances as delineated previously this effect is useful in the treatment of symptomatic hyponatremia resulting from water excesses

To produce a rise in serum Na concentration by osmotic diuresis calculations of the needed amount of mannitol first require estimation of the extent of negative water balance necessary It is essential that the intake component of this estimation include the volume of mannitol solution infused

*Table 2 Effect of diuresis on water and sodium excretion*

| PATIENT | BEFORE DIURESIS | | DURING DIURESIS | |
|---|---|---|---|---|
| DIURETIC AGENT | URINE FLOW ml/min | NA EXCRETION μeq/min | URINE FLOW ml/min | NA EXCRETION μeq/min |
| S E Mannitol | 0.40 | 2.20 | 1.76 | 6.31 |
| A L I Mannitol | 0.66 | 0.78 | 2.29 | 0.57 |
| A L II Mannitol | 0.18 | 0.30 | 1.88 | 1.20 |
| B S Mannitol | 0.38 | 1.10 | 2.18 | 1.88 |
| W R Mannitol | 0.28 | 3.10 | 1.58 | 18.2 |
| K O Mannitol | 1.08 | 1.60 | 1.98 | 1.67 |
| S E Hg | 0.36 | 1.75 | 2.14 | 187 |
| W R Hg | 0.80 | 6.01 | 1.79 | 372 |

Failure to do so may prevent an increase in serum Na level even though diuresis ensues (See Cases W R and K O)

After estimating the desired water loss this is converted to a urine flow rate (ml /min ) by relating it to the period of time over which the serum Na change is to occur After determining pre-diuretic urine flow (V) urine osmolality (U) and plasma osmolality (P) the number of milliosmols of osmotic diuretic required is then calculated in the following manner

$$\frac{UV}{P} = C_{osm} \text{ (pre-diuretic osmolar clearance)} \qquad (1)$$

Since at constant levels of ADH activity U and P remain constant substitution of the desired value V′ for V gives

$$\frac{UV'}{P} = C'_{osm} \text{ (osmolar clearance of diuresis)} \qquad (2)$$

$C'_{osm}$ may result from either an increase in V (V→V′) or in U (U→U′) therefore (2) may be rewritten

$$\frac{U'V}{P} = C'_{osm} \qquad (3)$$

$$\text{or} \quad U' = \frac{P \times C'_{osm}}{V} \qquad (4)$$

The requisite increase in milliosmolar excretion (I) is then

$$U' - U = I \text{ mOsm (needed for } V \rightarrow V') \qquad (5)$$

25 per cent mannitol contains approximately 1400 mOsm/L and thus

$$\frac{I\text{mOsm}}{1400} = \text{liters mannitol to be given} \qquad (6)$$

This is given intravenously at the rate of approximately 100 ml/hr so that dilution of mannitol in extracellular fluid and renal excretion will both occur at a rate sufficient to prevent extracellular fluid expansion with resultant pulmonary edema

Theoretical results obtained by fitting the data from these studies into the above calculations agree well with the observed changes within the range of experimental error And in further therapeutic trials in which the calculations have been carried out prior to mannitol administration the anticipated results have been observed

SUMMARY

Osmotic diuresis induced with mannitol has been shown to produce salt poor urine in clinical states in which renal sodium conservation is marked This effect is useful in the treatment of dangerous postoperative dilutional hyponatremia

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# FURTHER STUDIES IN BODY FLUID METABOLISM. II. THE RELATIVE EFFICACY IN MAN OF SODIUM BICARBONATE AND SODIUM LACTATE AS ALKALIZING COMPOUNDS, AND OF AMMONIUM CHLORIDE AND HYDROCHLORIC ACID AS ACIDIFYING COMPOUNDS*

JAMES D. HARDY, JOHN O. DAMPEER, JR. AND M. DON TURNER

Commercially prepared sodium lactate has long been the agent principally used intravenously to combat acidosis in clinical practice. Yet sodium bicarbonate would seem to have certain advantages over lactate and why the former has not been more frequently employed for this purpose has not been entirely clear. One prominent reason has been a common impression that a sodium bicarbonate solution could not be autoclaved without producing chemical alterations which rendered it unfit for intravenous use. Notwithstanding, since the purpose in using either compound in acidosis is to make available additional fixed base with as little other effect as possible, sodium bicarbonate would appear to be a more satisfactory substance: the $CO_2$ introduced is readily excreted by the lungs, whereas infused lactate ion must be metabolized. Thus the first part of this study consisted of a clinical comparison of sodium bicarbonate with sodium lactate.

The second portion of the study consisted of a clinical comparison of HCl with $NH_4Cl$ as acidifying agents. Despite the fact that in the literature it has frequently been stated that hydrochloric acid may also be used, it has been difficult to find physicians who did not prefer to employ ammonium chloride in the treatment of alkalosis—in fact few indeed appear to have used hydrochloric acid intravenously for this purpose. Nevertheless the infusion of $NH_4Cl$ does at times produce marked visual and gastrointestinal disturbances, and in the presence of liver failure the infusion of additional ammonium ion may be hazardous. In the present study the infusion of HCl in sodium chloride solution caused no symptoms, and the infusion of $NH_4Cl$ at the rate employed here occasioned no particular discomfort.

## METHOD

In the case of each of the four substances—sodium bicarbonate, sodium lactate, hydrochloric acid and ammonium chloride—167 mEq./1000 cc. were infused at a virtually constant rate over a period of 1½ hours. Blood samples were drawn before infusion, after ½ hour, after 1 hour and ½ hour after the end of the infusion. Measurements of blood pH (Beckman pH meter) and carbon dioxide content (Van Slyke manometric apparatus) were employed to calculate carbon dioxide combining power. Plasma chloride levels were determined, but despite individual changes the averaged experimental values for the groups did not vary significantly from the control levels.

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*From the Department of Surgery, University of Mississippi Medical Center, Jackson, Mississippi. This work was performed under Army Contract No. DA 49 007 MD 627, Office of the Surgeon General, Department of the Army.

With the technical assistance of Virginia Ward and Thelma Carter.

## RESULTS

Four groups of 10 patients each were studied. One group received sodium lactate, one sodium bicarbonate, one hydrochloric acid, and one ammonium chloride. Except for an occasional minor aberration, the subjects were in normal acid-base balance.

A representative set of data from each group is shown in Table 1. As was to be expected, the precise degree of change in any of the several values in a given individual varied considerably, but by averaging the changes for all patients it was found that sodium bicarbonate and sodium lactate were equally effective as alkalizing agents, and that hydrochloric acid and ammonium chloride were equally effective as acidifying agents. The infusion of $NaHCO_3$ and sodium lactate resulted in a rise in blood pH, plasma carbon dioxide combining power and total carbon dioxide content. The infusion of $NH_4Cl$ and HCl produced a fall in blood pH, a fall in plasma carbon dioxide combining power and a fall in total carbon dioxide content of blood. The plasma chloride level rose following the infusion of both $NH_4Cl$ and HCl, but, as anticipated, there was no consistent change in the chloride level following the infusion of sodium lactate and sodium bicarbonate.

All alterations in the measured values usually were progressive from the beginning of the infusion and had not returned to normal ½ hour after the end of infusion.

*Table 1. Chemical Changes in Venous Blood from a Representative Patient in Each Group*

| SOLUTION INFUSED | NAME | SAMPLE | pH | $CO_2$ CONTENT VOL. % | $CO_2$ CAPACITY mEq/L | Cl |
|---|---|---|---|---|---|---|
| $NaHCO_3$ | D.S. | C | 7.45 | 61.83 | 29.8 | 104.5 |
| | | 1 | 7.50 | 62.04 | 30.0 | 106.2 |
| | | 2 | 7.55 | 65.29 | 32 | 106.2 |
| | | 3 | 7.57 | 69.87 | 35 | 99.6 |
| Na Lactate | C.S. | C | 7.33 | 53.56 | 25 | 100.0 |
| | | 1 | 7.49 | 58.49 | 31 | 101.6 |
| | | 2 | 7.44 | 61.21 | 32 | 108.4 |
| | | 3 | 7.51 | 61.08 | 31 | 100.0 |
| HCl | M.I. | C | 7.40 | 60.83 | 24 | 103.6 |
| | | 1 | 7.35 | 55.90 | 23.6 | 112.3 |
| | | 2 | 7.37 | 50.97 | 21 | 111.6 |
| | | 3 | 7.37 | 47.68 | 21 | 108.7 |
| $NH_4Cl$ | I.W. | C | 7.40 | 58.25 | 28 | 108.2 |
| | | 1 | 7.35 | 53.56 | 27 | 112.2 |
| | | 2 | 7.32 | 45.56 | 24 | 116.5 |
| | | 3 | 7.31 | 45.50 | 24 | 112.2 |

# FURTHER STUDIES IN BODY FLUID METABOLISM II THE RELATIVE EFFICACY IN MAN OF SODIUM BICARBONATE AND SODIUM LACTATE AS ALKALIZING COMPOUNDS AND OF AMMONIUM CHLORIDE AND HYDROCHLORIC ACID AS ACIDIFYING COMPOUNDS*

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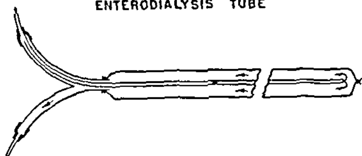



Fig 1 Fluid is perfused through the central plastic tube to the distal end of the cellophane tubing and back through the entire length of cellophane tubing to be collected at the outflow tube shown

well as that shown for introduction into the stomach or jejunostomy Using an automatic infusion-extraction apparatus with a program timer, it is possible to fill and empty the tube to collect samples, and to interrupt the infusion time at regular intervals for periods up to 24 hours

Thirty female dogs weighing 10 to 12 kg were used for the experimental preparations which consisted of left nephrectomy and either a Maydl jejunostomy or an isolated intestinal loop (Thiry Vella) fistula varying in length from 3 to 7 feet The second stage (right nephrectomy) procedure was performed through a flank incision about two weeks after the first operation when the dogs had regained normal health and weight Within 4 days the cellophane tube was introduced into the enteric fistula and perfusion of the lumen of the cellophane tube was begun using a perfusate free from K and N having the following composition Na 135 mEq/L Ca 5 mEq/L Mg 2 mEq/L Cl 110 mEq/L $HCO_3$ 15 mEq/L lactate 17 mEq/L and dextrose 0–10 gm/L The above amounts and flow rates were varied in accordance with specific experimental objectives Diet usually consisted of 25 per cent glucose solution Samples of the perfusate were taken from the cellophane tube at appropriate intervals and were analyzed for Na, K Ca Mg Cl $HCO_3$ P urea NPN, creatinine glucose pH and D O $Na^{24}$ $P^{32}$ and $K^{42}$ by standard methods with occasional minor modifications Blood was analyzed for the same constituents Deuterium oxide (heavy water) radiosodium[24] radiopotassium[42] and radiophosphorus[32] were administered intravenously and their rates of appearance were measured in the cellophane tubing In additional experiments these isotopes were introduced into the cellophane tube and the appearance rates in the blood were determined The kinetics of transfer of these respective substances in both directions between the blood and the lumen of the cellophane tube within the intestine were thereby evaluated

From the measured changes in concentration of the various substances in the dialysates and from the volume collected the total amounts of the several measured constituents removed or absorbed were calculated

## RESULTS

After determining the *in vitro* permeability characteristics of the cellophane to potassium and urea a closed cellophane tube was placed in the stomach of a normal human and the appearance half times of potassium and nitrogen were found to be about one hour with a similar finding when the cellophane tube was placed in the dog jejunostomy Instillation of D O $Na^{24}$ and $K^{42}$ directly into the small intestine with serial blood measurement showed an absorption half time of ten minutes while introduction of these

**Effect of Heat upon Sodium Bicarbonate Solution** No significant change was detected in sodium bicarbonate solution upon autoclaving

DISCUSSION

It is clear from these studies that intravenous sodium bicarbonate and sodium lactate solutions are approximately equally effective as alkalizing agents It is also clear that hydrochloric acid and ammonium chloride are both effective acidifying agents To avoid pain at the site of infusion the HCl solution must be rendered isotonic with additional solute (here NaCl) and to avoid distressing symptoms of nausea vomiting and visual disturbances the ammonium chloride must not be administered too rapidly

SUMMARY

1 Autoclaving sodium bicarbonate solution produced no chemical alterations that could be detected

2 Sodium bicarbonate and sodium lacetate proved equally effective alkalizing compounds Presumably therefore the more rapidly disposable anion of the former should render it superior to the latter for use in some patients in critical circumstances

3 Hydrochloric acid and ammonium chloride were almost equally effective acidifying agents When rendered isotonic by the addition of NaCl the HCl solution does not cause pain at the site of infusion In the presence of borderline hepatic function HCl would appear to possess certain theoretical advantages over $NH_4Cl$

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# ENTERODIALYSIS AN APPROACH TO THE PROBLEM OF RENAL SUBSTITUTION*

PAUL R SCHLOERB

In an attempt to combine the relative merits of intestinal perfusion and extracorporeal hemodialysis for removal of accumulated toxic metabolites in uremia a method using perfusion of a closed cellophane tube within the intestinal tract is being developed An interim evaluation of this method in the normal human nephrectomized dogs and one terminally uremic patient is described in this preliminary report

METHOD

A closed cellophane tube has been fashioned in various ways including that shown in Figure 1 Appropriate modifications have been employed to permit through and through perfusion within an isolated intestinal loop as

*From the Department of Surgery University of Kansas School of Medicine Kansas City Kansas, and the Kansas City Veterans Administration Hospital Kansas City Missouri
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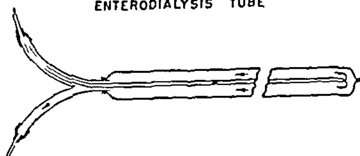



Fig 1 Fluid is perfused through the central plastic tube to the distal end of the cellophane tubing and back through the entire length of cellophane tubing to be collected at the outflow tube shown

well as that shown for introduction into the stomach or jejunostomy Using an automatic infusion-extraction apparatus with a program timer it is possible to fill and empty the tube to collect samples and to interrupt the infusion time at regular intervals for periods up to 21 hours

Thirty female dogs weighing 10 to 12 kg were used for the experimental preparations which consisted of left nephrectomy and either a Maydl jejunostomy or an isolated intestinal loop (Thiry Vella) fistula varying in length from 3 to 7 feet The second stage (right nephrectomy) procedure was performed through a flank incision about two weeks after the first operation when the dogs had regained normal health and weight Within 4 days the cellophane tube was introduced into the enteric fistula and perfusion of the lumen of the cellophane tube was begun using a perfusate free from K and N having the following composition Na, 135 mEq/L Ca 5 mEq/L Mg 2 mEq/L *Cl 110* mEq/L *HCO₃ 15* mEq/L *lactate 17* mEq/L *and dextrose* 0–10 gm/L The above amounts and flow rates were varied in accordance with specific experimental objectives Diet usually consisted of 25 per cent glucose solution Samples of the perfusate were taken from the cellophane *tube at appropriate intervals and were analyzed for* Na K *Ca Mg Cl,* $HCO_3$ P urea NPN creatinine glucose pH and D O $Na^{22}$, $P^{32}$, and $K^{42}$ by standard methods with occasional minor modifications Blood was analyzed for the same constituents Deuterium oxide (heavy water) radiosodium[22] radiopotassium[42] and radiophosphorus[32] were administered intravenously and their rates of appearance were measured in the cellophane tubing In additional experiments these isotopes were introduced into the cellophane tube and the appearance rates in the blood were determined The kinetics of transfer of these respective substances in both directions between the blood and the lumen of the cellophane tube within the intestine were thereby evaluated

From the measured changes in concentration of the various substances in the dialysates and from the volume collected the total amounts of the several measured constituents removed or absorbed were calculated

RESULTS

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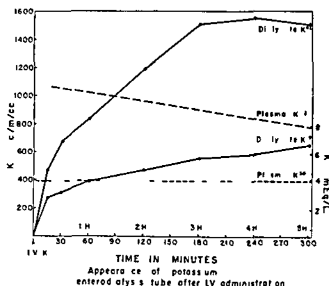



Fig 2 Appearance rates of radioactive and stable potassium in the cellophane tubing are shown. Volume measurements indicate that the higher concentrations of $K^{42}$ and $K^{39}$ in the tubing are not the result of water loss but reflect the normally increased concentration of potassium in the intestinal lumen.

isotopes into the closed cellophane tube within the intestine showed an absorption half time in the blood of about one hour. After intravenous administration of these isotopes (Fig 2) serial measurement of their appearance rates into a potassium and nitrogen free perfusate solution contained in the cellophane tube within the intestine showed an appearance half time of about 1½ hours indicating that the K, Na and $H_2O$ in the cellophane were in effect removed indirectly from the blood and not merely from the intestinal contents.

Metabolic balance data on normal and bilaterally nephrectomized dogs during enterodialysis indicated removal of potassium and nitrogen without marked change in sodium, Ca, Mg, Cl, $HCO_3$ or P. The rates of removal in uremia of potassium with cellophane tubing was 0.2–0.5 mEq/ft/hr while the non protein nitrogen removal rate was 63 to 103 mg /ft/hr. The maximum survival time of nephrectomized dogs without additional supportive treatment has been 11 days. Untreated controls survived 2 to 5 days.

One uremic patient, a 46 year old man with arteriolonephrosclerosis and malignant hypertension, was terminal and becoming moribund despite the usual medical supportive measures and was treated by enterodialysis using 7 feet of 27/32′ diameter cellophane introduced into the small intestine by jejunostomy under local anesthesia. Perfusion was begun (Fig 3) using an average volume of perfusate of 400 ml which was allowed to remain in the tube for an average of 150 minutes. This volume was alternately infused and aspirated for 63 hours when he expired suddenly from what proved at autopsy to be a large myocardial infarction. During this period 50 mEq of potassium and 14.7 gm of non protein nitrogen were removed without significant change in other electrolytes. Significant change in serum electrolyte and NPN values was not observed, although these latter data are not adequate for positive interpretation. Six hours after initiating enterodialysis reorientation and arousal from coma occurred. Of interest was the observation that at the shorter perfusion time (60 to 75 minutes) the removal of potassium and nitrogen were double the over-all average, corresponding to daily rates of removal of 36 mEq of potassium and 12 gm of nitrogen.

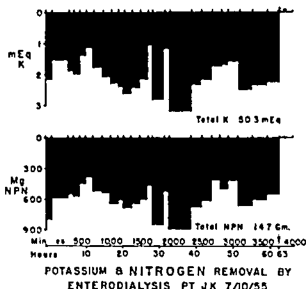



Fig 3 The width of each block represents the time during which the 400 ml of perfusate was allowed to remain in the cellophane tube The solution was then aspirated completely and the tubing was refilled with another 400 ml of K and NPN free solution Removal rate=mEq or mg/time Rate is greater at the shorter times

DISCUSSION

The formidable analytical problem of attempting to identify all or most of the accumulated metabolites in uremia makes it necessary to select certain entities which correlate with the severity of renal insufficiency Potassium non protein nitrogen urea and creatinine have been selected for study in these experiments with the realization that alterations in these substances may only indirectly reflect many other metabolic abnormalities

A relatively rapid exchange and transfer of potassium sodium and water from the blood to the lumen of the cellophane tube within the intestine has been demonstrated by isotopic and chemical methods Because of a similar appearance rate for non protein nitrogen urea and creatinine in the cellophane and absence of a decrease in this rate of appearance over a prolonged period it is assumed that a comparable transfer rate of NPN from blood to intestinal lumen exists Experimental evaluation of this assumption is in progress

The *transfer or exchange rate* of substances across the cellophane membrane within the intestine depends upon several factors including concentration gradient across the membrane temperature enteric pressure normal function of the intestinal epithelium presence or absence of intestinal obstruction (kinking or intussusception) and mesenteric blood flow The *amount* of substances exchanged or transported across the membrane is dependent upon both the exchange rate and the surface area Since the cellophane tubing diameter is constant for any given size of cellophane surface area may be expressed most simply as a function of length It is clear therefore that the amounts of metabolites removed are proportional to the length diameter and contained volume of the cellophane tube in addition to the factors previously mentioned

Problems encountered have included occurrence of intussusception in 30 per cent of the animals but not in the patient Development of anemia has been a limiting factor in survival studies and adequate dietary maintenance has been hindered by the uniform occurrence of vomiting while jejunostomy feeding has produced diarrhea

The metabolic balance data on the single uremic patient studied appears to indicate that amounts of potassium and nitrogen may be removed selectively in amounts in excess of the daily dietary requirement but present laboratory experience must be extended before clinical application can be considered in other than terminal renal failure due to irreversible kidney disease

SUMMARY

A new method using perfusion of cellophane tubing within the intestine for selective removal of toxic metabolites in uremia is being developed The dynamics of exchange between the blood and the lumen of the cellophane tube are presented Maximum selective daily removal of 36 mEq of potassium and 12 gm of non protein nitrogen was accomplished in one terminally uremic patient by this method

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## OBSERVATIONS ON THE ELECTROLYTE AND OSMOLAL CONCENTRATION CHANGES OF SERUM AND URINE IN RESPONSE TO VARIABLE CONCENTRATIONS OF INTRAVENOUS SODIUM CHLORIDE GLUCOSE AND WATER IN NORMAL SUBJECTS*

ROBERT L J BERRY, JOHN R ALGER VIVIAN IOB AND JAMES O ROBINSON

The response of the human kidney to the administration of parenteral fluids possesses great variability Particularly is this true when the renal excretion of intravenously administered water and sodium salts by the normal unstressed individual is compared to that observed in patients undergoing surgical operations The continuous oral or intravenous infusion of 5 per cent glucose and water consistently produces a negative water balance in the normal person [1] Under such conditions the urine volume may exceed the amount of infusate when as little as 100 ml /hr is given continuously for 24 hours Various explanations have been suggested to account for this phenomenon but none appear adequate, for it is probable that many factors are responsible [2 3 4] During operation and in the immediate postoperative period however the ability of the kidney to excrete comparable loads of water and salt may be sharply depressed [4 5 6] The overenthusiastic administration of both water and sodium salts during this period invites serious and even lethal postoperative complications

In the absence of significant extra renal loss of body electrolyte (e g gastro intestinal suction intestinal fistulae burns severe infections) the adminis

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tration of carbohydrate and water solutions for the provision of water needs during operation and in the immediate postoperative period has achieved wide acceptance Certain investigators however feel that a more physiological approach could be accomplished by the administration of hypotonic balanced electrolyte solutions during periods of maximum operative and postoperative stress

This report details initial studies done on normal human volunteers, further investigating the variable parameters of induced glucose and water diuresis and the effect upon this diuresis of hypotonic amounts of sodium chloride

METHOD

Seven normal volunteers were used in this study Because of space limitations only the results obtained in 6 studies of 2 members of the resident surgical staff will be presented

Without prior dietary control all subjects were fasted overnight A base line of rate of urine secretion and concentration was established by a specimen obtained during the 2 hours prior to starting the infusion The infusion period was 10 hours Subjects lay quietly in bed and stood erect only to void Hourly urine specimens were collected Venous blood samples were drawn at 0 3 7 and 10 hours Fasting continued for 14 hours following completion of the infusion Urine excreted during this period was collected and examined with the other specimens

With subjects serving as their own controls the infusions were repeated at periods varying from 3 to 7 days under comparable circumstances of environment and room temperature Relative humidity was not controlled The solutions used were 5 per cent glucose in water 0 15 per cent NaCl in 5 per cent glucose and 0 30 per cent NaCl in 5 per cent glucose solution Rates of administration were controlled by a constant infusion pump and varied from 204 to 264 ml /hr for the 10 hour period

Renal function was determined by 24 hour creatinine clearance test Sodium and potassium concentrations of serum and urine were determined by flame photometry Chloride was done by a modification of the Volhard procedure Serum proteins were determined by the Wolfson modification of the Weichselbaum technique [9] Voided urine specimens were frozen immediately and subsequent osmotic pressure determinations done with the Fiske Osmometer Serum osmolality was similarly determined

RESULTS

**Urine volume and excretion rates** Ignoring insensible loss subject Al was in negative water balance at the end of 24 hours regardless of the type of solution given (Fig 1) His largest negative balance was achieved with 0 30 per cent NaCl/glucose infusion Subject Ro had the largest negative balance of all subjects studied — 810 ml at the end of 24 hours when given glucose alone (Fig 2) He demonstrated a positive balance of 98 ml with 0 15 per cent NaCl/glucose and 373 ml with 0 30 per cent NaCl/glucose at the end of 24 hours If the insensible loss is estimated as 40 ml /hr it is evident that all studies ended in a net negative water balance regardless of the type of infusion given Maximum excretion rate was 7 ml /min This was observed during administration of glucose alone

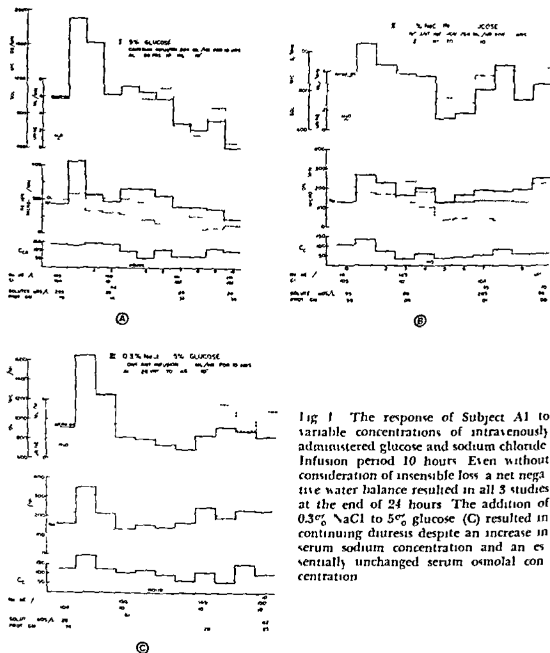



Fig 1 The response of Subject A1 to variable concentrations of intravenously administered glucose and sodium chloride Infusion period 10 hours Even without consideration of insensible loss a net negative water balance resulted in all 3 studies at the end of 24 hours The addition of 0.3% NaCl to 5% glucose (C) resulted in continuing diuresis despite an increase in serum sodium concentration and an essentially unchanged serum osmolal concentration

**Urine osmolality and electrolyte concentration** Except during the early period of the infusion and before the establishment of diuresis urine osmolality tended to vary inversely with volume The number of milliosmols of solute in urine excreted during the 10 hour infusion period showed remarkable constancy for all 3 test solutions Both subjects demonstrated a 5 per cent fall when 0 15 per cent NaCl/glucose was compared to glucose alone during the infusion period (Figs 1 & 2)

The highest hourly rates of excretion of sodium chloride and potassium were observed during the first hour of infusion with all test solutions in subject A1 After demonstrating a similar high level during infusion of glucose solution subject Ro achieved highest hourly excretion rates at 2 and 3 hours after initiation of infusion for 0 15 per cent NaCl/glucose and 0 30 per

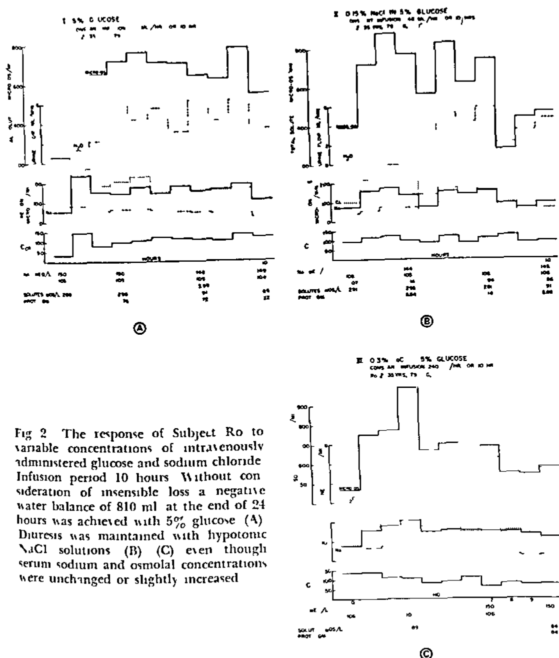


Fig 2 The response of Subject Ro to variable concentrations of intravenously administered glucose and sodium chloride Infusion period 10 hours Without consideration of insensible loss a negative water balance of 810 ml at the end of 24 hours was achieved with 5% glucose (A) Diuresis was maintained with hypotonic NaCl solutions (B) (C) even though serum sodium and osmolal concentrations were unchanged or slightly increased

cent NaCl/glucose respectively The excretion of sodium chloride and potassium demonstrated no striking changes during the infusion period when the 3 solutions were compared although there was a slight decrease in all 3 ions when 0 15 per cent NaCl/glucose solution was given There was a sharp increase in electrolyte excretion during the subsequent 14 hours after inclusion of NaCl in the infusate Both subjects excreted almost all of the load of NaCl during a 24 hour period when 0 15 per cent NaCl/glucose was given and subject Ro apparently had similarly excreted the salt load provided by 0 30 per cent NaCl Subject Al retained a part of the salt infused by 0 30 per cent NaCl/glucose as his excretion rates with this solution tended to parallel those of the more dilute saline solution

**Serum electrolyte concentration, osmolality and protein changes** In gen

eral the changes in serum sodium concentration paralleled those of osmolality but did not always occur in the same degree (Figs 1 & 2) Chloride and potassium changes were variable and appeared to be of little significance The decrease in serum osmolality and sodium levels was not so marked with 0 15 per cent NaCl/glucose as with glucose alone and during the administration of 0 30 per cent NaCl/glucose these changes were minimal Subject Ro apparently was able to diurese even though the osmolal concentration and sodium levels were above those observed at the beginning of the study Subject Al demonstrated a more conventional decrease in osmolal concentration and sodium When administered 0 30 per cent NaCl/glucose however, he was able to maintain diuresis despite an increase in serum sodium concentration no essential change in the serum osmotic pressure

Serum protein levels fell consistently regardless of the type of solution used At times these changes were marked being more than double the fall of serum sodium and osmolality This fall in serum protein occurred even when an increase in osmolality and sodium levels were found

## DISCUSSION

The addition of 0 15 per cent NaCl and 0 30 per cent NaCl to 5 per cent glucose did not prevent the negative water balance observed when 5 per cent glucose alone was given although the degree of negativity was less when salt was added During the period of infusion the number of mOsm excreted in the urine remained remarkably constant for all 3 test solutions During the subsequent 14 hours however the sodium load imposed by 0 15 per cent NaCl was essentially excreted and the load provided by 0 30 per cent NaCl was excreted in one subject and partially retained in the other

It has been stated that no other mechanism excepting the depression of antidiuretic hormone activity responds to hypotonicity of body fluids[9] If such is true then some other mechanism is responsible for the diuresis observed in certain of these studies that demonstrated little concomitant change in or actual increase of the serum osmolality and sodium concentration Certain reservations must be made however as hourly serum osmolal concentrations were not obtained

Welt and Nelson[9] observed in their studies that the fall in serum proteins accompanying administration of intravenous 5 per cent glucose and water to normal subjects did not occur when similar amounts of tap water were given by mouth As an explanation they suggested the excretion of electrolytes into the gastro-intestinal tract in response to a large volume of water The fall in serum proteins in these studies has been too great to be explained by simple dilution and furthermore occurred during periods of relatively increased sodium concentration and increased serum osmolality This suggests that proteins may have escaped in some manner probably into the interstitial fluids of the body If there is a relationship between such relative hypoproteinemia and diuresis of intravenous fluids under the conditions of these studies its confirmation awaits further investigation

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# THE TREATMENT OF EXPERIMENTAL CEREBRAL EDEMA BY INTRAVENOUS HYPERTONIC SOLUTIONS*

RAYMOND CLASEN SYLVIA PANDOLFI FELIX MARTIN AND C BRUCE TAYLOR

The role of cerebral edema in the basic patho physiology of cerebral trauma and vascular accidents has remained by and large unclarified and the treatment of cerebral edema by intravenous hypertonic solutions is still controversial This paper is an attempt to seek a partial answer to these questions by means of acute closed cerebral lesions produced by freezing through the intact skull The technique was originally described for rabbits [1] The work in this paper was done on dogs The lesions produced by this means consist of focal circumscribed areas of hemorrhagic tissue destruction involving the cerebral cortex (Fig 1) and extending for a variable distance into the adjacent white matter (Fig 2) Histologically there was complete destruction of neuronal elements with perivascular hemorrhage in the area of gross damage A complete description of the gross and microscopic characteristics together with the method for production of lesions in dogs has been published previously [2] Definite foci of cerebral edema were noted in the white matter adjacent to the areas of gross destruction This could be demonstrated both by ordinary histologic methods and by staining with vital dyes

## METHOD

Local closed cerebral lesions were produced in the posterior portion of the left hemisphere of adult dogs anesthetized with intravenous sodium pentobarbital The lesions were made by the application of isopentane frozen to the solid state by means of liquid air to the exposed intact skull The

*Rush Laboratories of Pathology and Surgical Research Presbyterian Hospital Chicago Illinois This work was supported in part by Contract AF16(600)628 monitored by The School of Aviation Medicine and in part by The Otho S A Sprague Memorial Institute



*Fig 1* A small lesion in the right hemisphere and a moderately large lesion in the left hemisphere produced by freezing through the intact skull The margins of the lesions are sharp allowing an accurate determination of the surface area

intracranial pressure was measured by means of a needle in the cisterna magna which was connected with a Statham strain gauge The animals were sacrificed at times varying from 150 to 600 min after the production of the lesion At the conclusion of each experiment the brain was removed and weighed The surface area of the lesion was determined by means of transferring a template to graph paper and counting the squares Each hemisphere was severed and re weighed separately The water content was determined by drying to constant weight in vacuo after acetone extraction The iron content of both the residue and extract was determined by the ortho



Fig 2 A cross section of the brain shown in Figure 1 The larger lesion extends for some distance into the white matter but the border is still well defined Edematous changes were noted in the white matter adjacent to the area of destruction

phenanthroline method after dry ashing with calcium carbonate.[3] The blood volume equivalent of this iron was then calculated by comparison with the concentration of iron in the venous blood just prior to the time the animal was sacrificed.

Five groups of animals were studied by this means. The first was a control group of 7 animals in which the normal weight, water content, and blood volume (expressed as the blood equivalent of hemisphere iron of canine cerebral hemispheres) was determined. The second group consisted of 9 animals with lesions in the left cerebral hemisphere. The third group consisted of 6 animals with unilateral left cerebral lesions treated by the intravenous administration of either 50 per cent glucose or 25 per cent human albumin. The fourth group consisted of 7 animals with unilateral cerebral lesions from 200 to 300 min. old treated with either 12 per cent or 20 per cent hypertonic dextran.[*] The lesion size, as determined by the surface area damaged per unit weight of the brain, was statistically the same at the 5 per cent level of probability for all groups. The fifth group consisted of 5 hemispheres removed from 4 normal anesthetized dogs that had been given intravenous hypertonic dextran.

RESULTS

The chemical results are given in Table 1 in terms of the means and estimated standard deviations of the groups. Student's "t" test with 5 per cent as the level of significance was used to compare figures. The results from Group 1 indicate that normal canine hemispheres from the same animal do not differ significantly from each other with respect to water and blood content as determined by the methods used.

*Table 1. Water and Blood Contents of Canine Cerebral Hemispheres*

| GROUP | WATER CONTENT (ml/100 gm TISSUE) | | BLOOD EQUIVALENT OF HEMISPHERE IRON (ml/100 gm TISSUE) | |
|---|---|---|---|---|
| | RIGHT | LEFT | RIGHT | LEFT |
| 1 | 78.53±0.42 | 78.37±0.39 | 4.72±0.35 | 4.65±0.28 |
| 2 | 78.61±0.29 | 79.55±0.33 | 4.55±0.38 | 8.10±0.66 |
| 3 | 78.20±0.54 | 79.76±0.51 | 6.71±0.23 | 9.45±0.94 |
| 4 | 80.25±0.49 | 81.52±0.50 | 6.52±0.62 | 8.62±1.14 |
| 5 | 78.91±0.70 | | 6.85±0.82 | |

In Group 2, animals with untreated left hemisphere lesions, a significant increase in blood and water content on the damaged side is seen. Comparison of the increments of blood and water in each animal indicates that they are independent variables ($r = 7.7 \times 10^{-4}$, showing no correlation). Furthermore, there was a direct relationship between the water increment in the damaged hemisphere and the size of the lesion ($r = 0.81$, significantly greater than zero at the 5 per cent level of probability) while no correlation was seen when the blood increment was compared to the lesion size ($r = 0.09$). The lesions were associated with an increase in intracranial pressure which

[*] The dextran was supplied through Dr. Homer Stavely of The Commercial Solvent Corporation.

varied from 120 mm to 480 mm and was sustained for as long as 10 hours While the pressure varied it never permanently returned to normal

The increased intracranial pressure was reduced by the use of hypertonic glucose Hypertonic albumin failed to reduce the intracranial pressure to the low levels obtained with glucose The chemical results indicate an increase in cerebral blood volume but no effect on either the water content of the undamaged hemisphere or the water increment in the damaged hemisphere (Group 3)

Following the use of hypertonic dextran (Group 4) there was a slight increase rather than decrease in intracranial pressure The chemical findings also indicate an increase in cerebral blood volume with no effect on the water increment in the damaged hemisphere There was however a significant increase in the water content of the undamaged hemisphere In order to determine whether or not this was due to the presence of the lesion dextran was given to normal animals There was little change in intracranial pressure The apparent difference in water content of the right hemisphere in Groups 4 and 5 is not statistically significant The effect of dextran on the water content of damaged tissue is apparently no greater than its effect on the water content of undamaged tissue

## DISCUSSION

The increment of water on a unit weight basis in the damaged hemisphere was relatively small but it must be emphasized that this is no measure of volume increase since it depends entirely upon the difference in water content of edema fluid blood and the brain substance in which these changes are taking place A better measure of the volume factor is the increase in total weight of the damaged hemisphere which averaged about 8 per cent while the average increase in water was only 1 per cent This over all increase in volume occurring locally in a closed system might be expected to have a considerable effect on intracranial dynamics and cerebral function

The effects of hypertonic fluids on the other hand might best be measured by considering the most labile component of cerebral edema namely its water content It has been shown in these experiments that hypertonic solutions have no effect on this factor The effect of all hypertonic solutions in increasing the cerebral blood volume is in keeping with the generally accepted notions on increased intravascular osmotic pressure and fluid movements Since the blood volume was increased and the water components of the brains were not affected the pressure reduction can only be explained on the basis of the Monro Kellie Doctrine[4] by assuming that spinal fluid was withdrawn from some reservoir not in the cerebral hemispheres

Dextran was not only ineffective in treating the localized cerebral edema in these experiments but its use led to a significant increase in the total water of the undamaged hemisphere Whether or not such a change has any physiologic significance remains to be determined On the basis of these findings we have come to the conclusion that cerebral edema of the type produced here is not diminished by intravenous hypertonic solutions Since dextran is often used to treat hemorrhage or shock in patients with intracranial injury our data also indicate that the potential danger of its use in such cases should be examined

CONCLUSIONS

1 Unilateral cerebral lesions produced by freezing through the intact skull in dogs are associated with an increase in blood and water in the damaged hemisphere and a state of intracranial hypertension

2 Hypertonic glucose and albumin reduced, temporarily the increased intracranial pressure even in the face of an increased intracerebral blood volume without affecting the increment of water in the damaged hemisphere and the water content of the undamaged hemisphere

3 Hypertonic dextran fails to reduce the increased intracranial pressure seen with these lesions and in addition causes a significant increase in the water content of the undamaged hemisphere without affecting the water increment of the damaged hemisphere The cerebral blood volume is increased to the same degree as seen with hypertonic glucose and albumin

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# INTERPRETATION OF THE SERUM POTASSIUM CONCENTRATION IN PATIENTS WITH ACID BASE IMBALANCE*

JAMES M BURNELL AND BELDING H SCRIBNER

It has long been suspected that acid base imbalances induce transfers of potassium between the cells and the extracellular space However there are conflicting opinions as to the direction and magnitude of these transfers Inasmuch as clinicians must of necessity use the serum potassium concentration as a guide to potassium therapy it is of importance to clarify the direction and magnitude of change in serum potassium concentration induced by alteration of extracellular pH The present study of 5 human subjects demonstrates that acidosis increases and alkalosis decreases the serum potassium concentration independently of changes in total body potassium

* From the Veterans Administration Hospital and the Department of Medicine University of Washington School of Medicine Seattle Washington Supported by grants from Abbott Laboratories the American Heart Association the National Institute of Health (Grant #H 2185) and the Washington State Heart Association

METHOD

Five patients were selected for study because of alterations of extracellular pH of either metabolic or respiratory origin. Extracellular pH was manipulated by infusions of $NaHCO_3$, $NH_4Cl$, HCl and on one occasion the concommitant use of 8 per cent $CO_2$ inhalation. Effort was made during the period of pH change to minimize changes in the body potassium as related to nitrogen.

Whole blood pH was measured anaerobically on femoral venous blood within 10 minutes after sampling. pH measurements were made at 38°C using a Cambridge pH meter with glass electrode. Venous rather than arterial pH was determined because central venous pH was considered to more accurately reflect extracellular pH. Plasma potassium concentrations were measured on the same specimens using a Baird flame photometer with lithium internal standard. Potassium and nitrogen balances were measured by standard methods.

RESULTS

The effect of extracellular pH change on the serum potassium concentration is illustrated by the course of events in patient H. M. (Fig. 1). The patient had respiratory alkalosis associated with hepatic coma. Extracellular pH was manipulated by a combination of HCl infusion and carbon dioxide inhalation. No potassium was given during the 5 day study. The reciprocal relationship between extracellular pH and serum potassium concentration is apparent throughout the study.

Data from 9 experimental periods of study of 5 patients are presented in Table 1. In all instances acidosis increased and alkalosis decreased the serum potassium concentration. Changes in total body potassium in excess of nitrogen were small (Column 5 of Table 1). Furthermore, in 6 of the 9 periods of study the change in potassium balance was in the opposite direction to the change in the serum potassium concentration. Thus, changes in potassium balance were not the cause of the observed changes in serum potassium concentration.

In the last column in Table 1 change in serum potassium concentration has been calculated for each 0.1 unit pH change in the extracellular space.

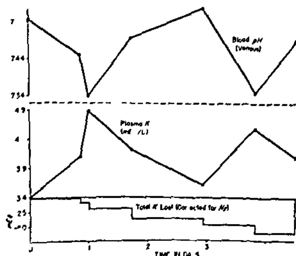



Fig. 1. Serial changes in blood pH, plasma potassium concentration and potassium balance in patient H.M.

*Table 1 Relationship Between Changes in Extracellular pH and Change in Serum Potassium Concentration in 9 Experimental Periods in 5 Human Subjects*

| PATIENT | PERIOD | ΔK | ΔpH | $\Delta K_b$ | ΔK/0.1ΔpH |
|---|---|---|---|---|---|
| H M | 1 | +0.7 | −0.10 | −4 | 0.70 |
| | 2 | +1.0 | −0.10 | −6 | 1.00 |
| | 3 | −0.9 | +0.16 | −10 | 0.56 |
| | 4 | −0.6 | +0.08 | −18 | 0.75 |
| | 5 | +1.0 | −0.23 | −6 | 0.44 |
| J R | 1 | +0.5 | −0.13 | −5 | 0.39 |
| H I | 1 | −2.4 | +0.48 | +32 | 0.50 |
| J N | 1 | −1.9 | +0.15 | 0 | 1.27 |
| J D | 1 | −2.4 | +0.47 | −15 | 0.51 |

$\Delta K_s$=Change in the serum potassium concentration in mEq/L
$\Delta K_b$=Change in potassium balance in excess of nitrogen (K/N ratio=3mEq/gm)

The mean change for all periods was 0.68 mEq/L change in serum potassium concentration per 0.1 unit change in extracellular pH. The range was 0.39 mEq/L to 1.27 mEq/L change in the serum potassium concentration per 0.1 unit pH change.

DISCUSSION

The serum potassium concentration in the absence of acid base imbalance accurately reflects intracellular potassium stores[1] Alkalosis decreases and acidosis increases the serum potassium concentration in relation to the intracellular stores. The greater the deviation of extracellular pH from normal the more the serum potassium concentration fails to reflect the intracellular stores. This deviation may be so great that in severe alkalosis there may be a very low serum potassium concentration with essentially normal potassium stores. In severe acidosis a high serum potassium reflects normal potassium stores, a normal serum potassium reflects a moderate potassium depletion and a low serum potassium reflects a profound depletion. The muscle analyses of Mudge and Vislocky offer support for these concepts.[2]

If the serum potassium concentration is to be interpreted to reflect the level of intracellular potassium stores either there must be no acid base disorder present or correction must be made for deviations of up to 3 mEq/L in the serum concentration which can be induced by alteration of extracellular pH.*

Although the serum potassium concentration fails to reflect potassium stores in the presence of acid base imbalance, it remains the best practical guide to therapy. Whether initial *hypokalemia* is secondary to intracellular potassium depletion, alkalosis or both, potassium chloride is good therapy. If potassium depletion is the dominant cause of the hypokalemia, potassium is needed. If alkalosis is the dominant cause of hypokalemia, potassium chloride will help compensate for increased renal excretion of potassium which may accompany alkalosis[3] also provision of chloride ion even though the

* The effect of alterations of extracellular pH on the relationship between extracellular and intracellular potassium is probably related to intracellular buffering and therefore ultimately dependent upon intracellular pH change. However since the latter cannot be measured the relationship must be studied in terms of extracellular pH.

potassium may be excreted will aid in the repair of the alkalosis Conversely whether initial *hyperkalemia* is secondary to acidosis intracellular potassium excess or both potassium restriction would be indicated because of the danger of producing cardiotoxicity Restriction is also indicated because hyperkalemia would represent at most minimal potassium depletion even with severe acidosis However it is important to again emphasize that the more severe the acidosis the less the serum potassium concentration reflects the intracellular stores Thus in the presence of severe acidosis a normal initial serum potassium concentration represents significant depletion and warrants potassium therapy as soon as an adequate urine volume permits Rapid correction of the acidosis such as occurs in the treatment of diabetes acidosis causes a rapid fall in the serum potassium concentration This fall makes early therapy with potassium perfectly safe and gives additional warning of potassium depletion

The results of this study have at least two additional implications 1) Extracellular pH must be controlled in all experiments investigating other factors which may change the serum potassium concentration 2) Avoidance or correction of acidosis will aid in control of hyperkalemia in patients with renal failure

SUMMARY

1 The effect of acid base imbalance on the serum potassium concentration has been studied in 5 patients

2 Acidosis increases and alkalosis decreases the serum potassium concentration independently of changes in total body potassium

3 These observations permit more accurate estimation of intracellular potassium stores from the serum potassium concentration in patients with disturbance of acid base imbalance

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# METABOLIC STUDIES ON INFANTS WITH ENTEROSTOMIES*

WILLIAM R RICHARDSON

The use of enterostomy in the management of small bowel obstruction requiring resection in infants has been frequently condemned but its revival as part of a Mikulicz type two stage procedure during the past 7 years at Boston Children s Hospital has been associated with a gratifying increase in survivors Most surgeons have avoided its use even as a temporary measure because of disastrous losses of fluid and electrolytes especially in the newborn infant We have found that large enterostomy losses can be adequately replaced and need not constitute a contraindication to the use of this technique The purpose of this study was to define the patterns of enterostomy function and the extent of the gastrointestinal losses of fluid and electrolyte Time does not permit discussion of other aspects of nutritional problems and care here

## METHOD

Nutritional data have been recorded on the 180 cases of small bowel obstruction managed by this method in the past 7 years Most of the patients were under 1 year of age and over half were newborn Diagnoses were congenital atresia 50 meconium ileus 10 intussusception 30 congenital stenosis 12 volvulus perforated Meckel s diverticulum duplication hernia neoplasm and miscellaneous totalling 48 others Many serum chemistries extrarenal loss analyses and spot observations on gastro intestinal function were made and a detailed metabolic balance was performed on an infant with congenital ileac atresia Representative charts have been studied extensively with calculations of losses based on theoretical considerations as well as measured intake and body weight

The balance and analytic techniques employed were those of the Children s Hospital Metabolic Unit under the supervision of Dr William M Wallace of the Department of Pediatrics † Collection and measurement procedures have been described elsewhere [1] Ileostomy losses were measured by gravimetric technique for volume and water content and electrolyte losses were calculated by analyses of the equilibrated wash fluid Recoveries by this method were accurate within 3 per cent for inorganic ions

Balance analyses were performed as follows Sodium and potassium in 0 2 ml serum or 1 0 ml urine by flame photometry[2] chloride in stool drainage and urine by a modification of the method of Wilson and Ball[3] and in serum by the method of Van Slyke and Hiller[4] blood pH by the method of Sendroy and Hastings and total carbon dioxide by the method of Van Slyke and Neill [6]

## RESULTS

Figures 1 and 2 present balance data on infant R S from a 12 day period beginning the day after ileostomy and ending 4 days after closure when normal stools were appearing

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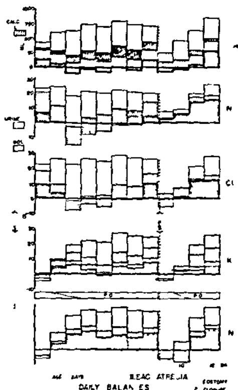



Fig 1 Daily sodium chloride potassium nitrogen and fluid balances in patient R S Urinary and ileostomy (stool) losses are charted downward from the total intake line Positive balances are the dark areas above and negative balances everything below the zero line Column width has no significance Daily fluid balance minus calculated insensible weight loss (1000 gm /M /day)[7] equals theoretical body weight change (not corrected for water content)

R S had lost 13 per cent of his birth weight of 3 1 kg by the time of admission at age 9 days At operation a short atretic distal ileac segment was resected The ileostomy spur was crushed by the eighth day and extraperitoneal closure under cyclopropane anesthesia was performed on the ninth day He was discharged home 13 days later weighing slightly more than birth weight Oral intake was entirely Nutramigen* after the third day Extrarenal losses were almost entirely intestinal and insensible

Nitrogen losses were small and balance was soon steadily positive Only water and electrolytes need concern us in such an acute fluid disturbance

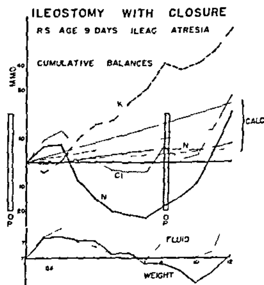



Fig 2 R S cumulative balance and body weight curves compared with theoretical normal growth retention curve

*Nutramigen—a casein hydrolysate mixture Mead Johnson & Co

Daily electrolyte losses via ileostomy were remarkably similar even with varying volumes. Measured extrarenal sodium and potassium losses averaged 45 per cent of total output after the first day contrasted with 26 per cent for water, 28 per cent for chloride, and 25 per cent for nitrogen. The briefly positive sodium and chloride balances in the beginning and preclosure days reflect decreased urinary losses.

Figure 2 attempts to present a more composite picture by charting cumulative balance curves which have proved of special value in studies of rapidly growing infants. No conclusions can be drawn concerning the metabolic response to the first operation, but certainly the ileostomy closure 9 days later constituted minimal biological stress.

R.S. had a more uneventful convalescence than the average. His low ileostomy demonstrated fairly effective water and electrolyte absorption, but his renal losses, especially of water, were excessive, perhaps due to immaturity.

Figure 2 also indicates the pattern and magnitude of the water exchanges. The calculated and observed body weight curves coincide remarkably except for a decrease in the measured urinary output on the day before closure.

The ileostomy drainage volume levelled off under 200 ml. on exclusively oral intake (Table 1). It contained more sodium than chloride but was hypotonic for both. The potassium content was consistently high, with values 5 to 6 times the extracellular fluid concentration and about 1¼ times the intake concentration.

*Table 1. Composition of Gastrointestinal Loss*

| SOURCE | DAILY VOLUME | CONCENTRATION IN mEq./L. Na | K | Cl |
|---|---|---|---|---|
| R.S. Day #2—Ileostomy | 70 | 152 | 81 | 54 |
| R.S. Day #3—Ileostomy | 133 | 94 | 50 | 42 |
| R.S. Day #4—Ileostomy | 151 | 73 | 36 | 32 |
| R.S. Day #5—Ileostomy | 132 | 83 | 56 | 39 |
| R.S. Day #6—Ileostomy | 194 | 56 | 50 | 37 |
| R.S. Day #7—Ileostomy | 185 | 51 | 46 | 34 |
| R.S. Day #8—Ileostomy | 140 | 67 | 48 | 49 |
| R.S. Average Days 4 thru 8 | 160 | 66 | 47 | 38 |
| Tube suction drainage (stomach) (infants under 6 months) | 12 specimens patients | 73 (50-100) | 11 (3-21) | 93 (50-114) |
| Tube suction drainage (jejunum) (infants under 6 months) | 8 specimens patients | 94 (80-106) | 3.1 (2.4-5) | 94 (87-102) |
| Oral intake—Nutramigen (average of several batches) | | 29 | 31 | 35 |
| Evaporated milk (modified) formula | | 26 | 30 | 31 |

In a large number of cases of various types with varying patterns of intestinal function, theoretical volumes and sodium contents of enterostomy drainage have been calculated. Table 2 presents data from 9 representative cases. The first infant should represent some kind of a record water turnover or exchange rate. During his wettest consecutive 4 day period his average

*Table 2 Representative Cases—Intake and Output Data*

| INITIAL | C R | D O | L T | P F | J P | W B | A G | P.A | E C | |
|---|---|---|---|---|---|---|---|---|---|---|
| AGE DAYS | 60 d | 1 | 9 | 2 | 3 | 3 | 1 | 75 | 1 | |
| WEIGHT kg | 3.3 kg | 3.2 | 2.2 | 2.4 | 2.5 | 3.2 | 2.4 | 4.0 | 2.7 | |
| DIAGNOSIS | LOW ILEAL STENOSIS | HIGH IL ATRESIA | MID IL ATRESIA | LOW JEJ ATRESIA | HIGH IL ATRESIA | MID IL ATRESIA | MECON ILEUS | LOW IL STENOSIS | MEC ILEUS | AV |
| Length of Period A* days | 4 | 3 | 11 | 3 | 3 | 4 | 3 | 4 | 4 | 4+ |
| Length of Period B† days | 12 | 1 | 8 | 9 | 4 | 17 | 6 | 9 | 8+ | 8+ |
| INTAKE | | | | | | | | | | |
| Rate Ml/kg/d | 396 | 342 | 312 | 260 | 183 | 182 | 177 | 170 | 151 | 241 |
| Rate ml M²/d | 6900 | 5080 | 4560 | 3950 | 2860 | 2820 | 2560 | 2890 | 2400 | 3780 |
| % Parenteral | 43 | 26 | 43 | 31 | 0 | 10 | 9 | 1 | 0 | 18 |
| Average Na conc | 52 | 46 | 39 | 38 | 26 | 28 | 35 | 25 | 26 | 35 |
| ENTEROSTOMY OUTPUT‡ | | | | | | | | | | |
| Rate ml/d | 1000 | 595 | 385 | 315 | 184 | 157 | 118 | 211 | 70 | 337 |
| Rate ml/kg/d | 310 | 185 | 176 | 130 | 74 | 50 | 49 | 53 | 25 | 117 |
| Rate ml/M²/d | 5000 | 2550 | 3340 | 2000 | 1150 | 830 | 710 | 910 | 405 | 1900 |
| Average Na Conc. | 51 | 65 | 57 | 53 | 39 | 58 | 85 | 47 | 83 | 60 |

*Period A is early postoperative before full G I function established

†Period B follows till ileostomy closure (functional plateau)

‡Output values are calculated assuming liberal daily average skin lung and kidney expenditure of $H_2O$=2000 ml/M² Na=30 mEq/M²

daily intake was 514 ml /kg or 8770 ml /M (13 per cent parenteral) with average sodium concentration This translates to an enterostomy loss of 1370 ml /day or 400 + ml /kg /day The adult equivalents calculated on any basis are startling

Most of the survivors in the newborn group fitted a generally similar clinical pattern An average case would show 1 Nasogastric tube suction 1–2 days 2 Full oral formula by the fourth or fifth day 3 Full parenteral intake for 2 to 3 days tapering to an occasional supplement after full enterostomy function was established 4 Small gradual loss of weight reversed after oral intake was resumed following closure with discharge weight about equal to birth weight 5 Mild physiological disturbance by operative closure on the 7 to 12th day 6 Enterostomy output volume plateau of 250–300 ml /day (equivalent to approximately 100 ml /kg or 1500 ml /M²/day) 7 Enterostomy drainage concentrations averaging in mEq/L Na 60 70 Cl 45 50 K 35-40 8 Average daily enterostomy losses (plateau) 250 gm of water 16 mM of sodium 12 mM of chloride and 9 mM of potassium 9 Intestinal transit times (carmine) averaged about 1 hr with a range of 10 min to 2½ hr

## DISCUSSION

The volume and composition of these enterostomy losses are suggestive of those recorded for infants with moderately severe diarrhea Although a few spot analyses of drainage from parenterally fed infants show a composition similar to that of orally fed infants and it is known that stools from fed and fasted infants with diarrhea are generally similar comparable complete balances are needed to permit evaluation of the role of intake in the determination of the enterostomy loss The wide range of losses points up the importance of frequent quantitative checks on clinical observation These can usually be chiefly intake volumes and body weights but the occasionally severely ill or profusely draining infant will need methods for volumetric collection of urine weighing of ileostomy dressings and even electrolyte analyses of loss samples

The commonest metabolic imbalance encountered in the young infants was hypertonic dehydration due to a relative water deficit probably occurring through the combined effects of low intake and loss of hypotonic fluid from skin lungs and intestinal tract

## SUMMARY

1 Enterostomy losses in infants need not be disastrous

2 Although marked variations occur a general pattern of clinical gastrointestinal function has characterized the survivors

3 Extrarenal loss analyses have confirmed the generally hypotonic nature of infant gastro intestinal secretions and have re emphasized the value of frequently weighing the infant and his output

Acknowledgement I am grateful for the opportunity and encouragement provided by Dr Robert E Gross on whose surgical service these patients were studied during my periods of residency and research fellowship

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# THE EFFECT OF 19 NORTESTOSTERONE CYCLOPENTYLPROPIONATE ON NITROGEN BALANCE AND BODY WEIGHT IN POSTOPERATIVE PATIENTS*

WILLIAM E ABBOTT STANLEY LEVEY HARVEY KRIEGER JERREI W BENSON AND C JACKSON RAYBURN

The large nitrogen deficits which have been reported to occur in surgical patients maintained on a starvation regimen (e g 1 to 3 L of 5 to 10% hexose solution/day) can be minimized or even negated by giving increased amounts of both nitrogen and calories [1] In an individual of small stature (40 kg or less) it is possible to administer a sufficient caloric (35 to 40) and nitrogen (0 25 to 0 3 gm ) intake on a per kg basis by using 2500 to 3000 ml of a 5 per cent protein hydrolysate solution containing 10 per cent hexose However it is difficult to meet even the minimal caloric requirements (25 to 30 calories/kg /d ) of the adult surgical patient who weighs more than 50 kg by use of only protein hydrolysates and sugar solutions It has been shown previously[1 2 3] that nitrogen wasting and weight loss can be minimized in the postoperative patient by administering a fat emulsion intravenously as a source of additional calories But since intravenous fat emulsions are not readily available at this time the possibility was investigated of achieving reduced nitrogen deficits in surgical patients by giving them adequate amounts of nitrogen and modest caloric intakes (15 to 25 calories/kg of body weight) plus an anabolic hormone 19 Nortestosterone Cyclopentylpropionate †

Metabolic balance studies were carried out on 13 gastrectomized patients 7 males and 6 females who were maintained on adequate nitrogen and modest caloric intakes Two of the males and 3 females also received an intra

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*From the Department of Surgery Western Reserve University School of Medicine and the University Hospitals of Cleveland Cleveland Ohio This work was supported in part by grants from the National Institutes of Health U.S Public Health Service (A 760 (C3) the Baxter Laboratories Inc Morton Grove Illinois The Upjohn Company Kalamazoo Michigan and the John A Hartford Foundation

†19 Nortestosterone Cyclopentylpropionate (Depo Nortestonate) was supplied by The Upjohn Company Kalamazoo Michigan

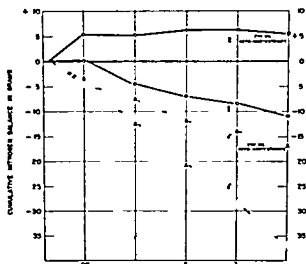


Fig 1 The average cumulative daily nitrogen balance for 7 male and 6 female subtotally gastrectomized patients who were maintained on comparable caloric and nitrogen intakes Two of the males and 3 of the females received 250 mg of Depo nortestonate

muscular injection of 250 mg of Depo-nortestonate 1 or 2 days before operation The details of the methods used in carrying out the metabolic balance studies have been reported elsewhere [1 3]

RESULTS

The average 5 day cumulative nitrogen balance including the day of operation and next 4 postoperative days for these 13 patients are shown in Figure 1 The 7 male patients were all given 0 15 to 0 2 gm of nitrogen and 20 to 25 calories/kg of body weight

The 5 male patients who did not receive the Depo nortestonate showed 5 day cumulative nitrogen deficits ranging between 33 7 and 39 4 gm or an average deficit of 36 7 gm The postoperative temperatures of these 5 patients were essentially normal The 2 patients who received 250 mg of Depo-nortestonate preoperatively had only a 15 and 18 gm nitrogen deficit during this 5 day period in spite of pulmonary atelectasis and a fever of 38 to 39°C which persisted for 3 and 4 days The average 5 day cumulative nitrogen deficit for these 2 patients was 16 7 gm The 5 men who were maintained on modest caloric and nitrogen intakes lost between 3 to 5 pounds by the fifth postoperative day The 2 men who had comparable operations and nutritional intakes and also received Depo nortestonate (250 mg) showed no significant change in their weight postoperatively

The average 5 day cumulative nitrogen deficit for the 3 female patients who had modest caloric (20 to 30 cal /kg ) and nitrogen intakes (0 2 to 0 3 gm/kg ) was 10 7 gm In contrast there was an average 5 day cumulative positive nitrogen balance of 5 4 gm for the 3 females who received Depo nortestonate The 3 women who received the modest caloric and nitrogen intakes lost from ½ to 3 pounds while those on a similar regimen plus Depo-nortestonate gained 1 to 3 pounds over the 5 day postoperative period The sodium and water balances in these patients revealed that the weight changes were not related to gains or losses of these substances The sodium intake had been restricted to from 50 to 70 mEq in these patients and the 5 day cumulative sodium balances showed only slight deviations from equilibrium In several other patients not included in this report who received depo nortestonate and 100 to 155 mEq of sodium daily positive sodium balances of from 20 to 60 mEq daily occurred for the first 6 to 9 postoperative days These stud

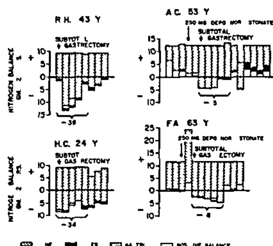


Fig 2 Nitrogen balance studies in 4 subtotally gastrectomized male patients The degree of negative nitrogen balance is expressed by the extent of the column below the zero or base line The cumulative 5 day postoperative nitrogen deficit is shown for each patient below the individual balance charts

ies indicate that the partially gastrectomized patients who received modest caloric and nitrogen intakes showed smaller nitrogen deficits if they were given 250 mg of a long acting anabolic hormone (Depo nortestonate) postoperatively

Figure 2 shows nitrogen balance charts for 4 of the male patients 2 patients (F A and A C) received the Depo nortestonate The caloric and nitrogen intakes per kilogram of body weight per day were essentially the same for all 4 of these patients It should be noted in the nitrogen balance chart of H C that this patient consumed orally significantly less nitrogen than he received intravenously for the first 5 study days This diminished nitrogen intake was a result of his inability to eat more and this in turn prolonged the period of negative nitrogen balance It has been noted that many patients are unable to take adequate oral diets during the first 5 to 10 postoperative days " [3] On the other hand the patients who received the Depo-nortestonate usually did not have postoperative anorexia

DISCUSSION

The female patients had smaller nitrogen deficits than the male patients even though their nutritional regimens (per kg of body weight per day) were comparable Similar findings were also noted in a previous study of subtotally gastrectomized patients who received no nitrogen and only a hexose solution for the first 5 postoperative days [1] The male patients on such a starvation regimen had an average cumulative 5 day nitrogen deficit of 56 6 gm as compared to 30 0 gm for the female patients who likewise underwent a subtotal gastrectomy and received only 150 to 300 gm of hexose daily Because of this apparent difference between males and females in their response to trauma one is not justified in comparing the nitrogen deficits of male postoperative patients with those of female postoperative patients

No untoward effects were noted which could be attributed to the anabolic hormone The female patients showed no androgenic effects No accumulation of water and salt was noted if the sodium intake did not exceed 60 mEq /d

SUMMARY AND CONCLUSIONS

From the data presented it is apparent that it is possible to reduce or negate the nitrogen deficit in the postgastrectomized patient by giving moderate

amounts of nitrogen and calories and employing an anabolic hormone such as Depo nortestonate

Male patients show a larger nitrogen deficit following surgery than do female patients undergoing similar operations when both groups are maintained on comparable intravenous regimens expressed on a per kilogram of body weight basis

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# EFFECTS OF THERMAL INJURY ON METABOLIC RATE AND INSENSIBLE WATER LOSS IN THE RAT*

ZELIG H LIEBERMAN AND JAMES M LANSCHE

This study was undertaken to investigate the influence of thermal injury upon the interrelationship of the metabolic rate and insensible loss of water in the burned rat The rate of insensible loss of weight of rats bearing full thickness burns of 5 to 20 per cent of body surface significantly exceeds normal[1]

## METHOD

Eighteen adult male Sprague Dawley strain rats were studied Triweekly 1 hr measurements of oxygen consumption carbon dioxide production and insensible water loss were performed upon rats fasted for 12 hr using a Benedict indirect calorimeter[2] The heat production expressed as calories per square meter of body surface for 24 hr was calculated from the measured oxygen consumption Heat lost through the vaporization of water was determined by converting the measured extrarenal excretion of water to heat equivalents by employing the conversion factor of 586 calories/gm water Following a 2 week control period full thickness dorsal burns of 5 to 20 per cent of body surface were produced with radiant energy from a parabolic reflector while the animals were anesthetized with ether The rates of healing of the wounds were determined by the Carrel method[3] Rectal cutaneous and wound temperatures were recorded with thermistors

* From the Department of Surgery Washington University School of Medicine St Louis Missouri Based upon studies conducted under Army Contract No DA 49 007 MD 636 Office of the Surgeon General

The hair upon the dorsal surfaces of the body was clipped closely before the control period of observation

The metabolic disturbances associated with a simple open wound were studied after the excision of skin from 10 to 30 per cent of the dorsal surface of the bodies of 3 anesthetized rats

RESULTS

The metabolic rate ($O_2$ consumption ml /sq m /d ) and insensible water loss (gm /sq m /d ) were essentially constant for individual rats throughout the control period Heat lost through vaporization of water represented 21 per cent ± 2 of the total heat produced (See Table 1) This agrees very closely with Benedict s observations[4]

Oxygen consumption and the rate of insensible water loss had fallen significantly by the third hour after injury This decrease in oxygen consumption and insensible loss of water were directly related to the magnitude of the burn (The larger the injury the greater the decline in metabolism and water loss )

Between the second and seventh post burn days the insensible loss of water and oxygen consumption rose above pre burn levels respectively attaining rates of 40 to 60 and 10 to 15 per cent above pre burn levels with burns covering 15 per cent of the rat Concomitantly the proportion of heat lost through the vaporization of water increased from the control value of 21 per cent to 30 to 40 per cent of the total heat produced (Table 1) Associated with the separation of the eschar and the conversion of the injury to an open wound the insensible loss of water and metabolic rate increased further and attained respective levels of 100 to 150 per cent and 15 to 25 per cent above the pre burn levels (Table 1) While the open wound was large the heat lost by vaporization amounted to 40 to 60 per cent of all the heat produced As the wound healed the rates of insensible loss of water and metabolism returned to the control levels

There was no significant difference in rectal temperature during the pre burn and post burn periods except for a transient decrease in temperature noted immediately after thermal injury in the rats with the most severe burns The surface temperature of the eschar was 0 8°C ± 0 2 and of the open wound 1 0°C ± 0 2 less than the surrounding skin

One rat with a 15 per cent thermal injury was studied to determine the effect of placing an impermeable dressing (Saran wrap) over the burn eschar Coverage of the eschar with Saran wrap was attended with an immediate decrease of the insensible water loss and metabolic rate to normal pre burn levels (Table 2)

**Influence of Skin Excision** Open wounds of 10 per cent body surface were produced in 2 rats There was an immediate three fold elevation of rate of insensible water loss and a one fourth fold increase in metabolic rate and the heat loss by vaporization of water amounted to about 70 per cent of total heat produced Coverage of these open wounds with the skin removed previously was associated with an immediate return of the rates of insensible water loss and oxygen consumption to normal levels (Table 3 Rats A and B)

Excision of 30 per cent of the skin was followed by death of the rat within 18 hr In this experiment the insensible water loss rose immediately and

*Table 1 15% Burns Comparison of Total Heat Production and Heat Loss Through Water Vaporization in the Burned Rat*

| | CONTROL | | | IMMEDIATE POST BURN | | | ESCHAR | | | OPEN WOUND | | | HEALED | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ANIMAL | TOTAL HEAT (A) | HEAT VAP $H_2O$ (B) | HEAT VAP % T (C) | TOTAL HEAT | HEAT VAP $H_2O$ | HEAT VAP % T | TOTAL HEAT | HEAT VAP $H_2O$ | HEAT VAP % T | TOTAL HEAT | HEAT VAP $H_2O$ | HEAT VAP % T | TOTAL HEAT | HEAT VAP $H_2O$ | HEAT VAP % T |
| 1 | 912 | 233 | 25 1 | 820 | 205 | 25 0 | 1022 | 398 | 38 5 | 1159 | 508 | 43 9 | 985 | 240 | 24.3 |
| 2 | 9[illegible]4 | 230 | 23 7 | 838 | 230 | 27 5 | 1029 | 380 | 37 0 | 1077 | 539 | 50 1 | 988 | 263 | 26 6 |
| 3 | 899 | 225 | 25 2 | 720 | 185 | 25 7 | 1091 | 425 | 38 9 | 1104 | 578 | 52.3 | 956 | 246 | 25 7 |
| 4 | 995 | 237 | 23 2 | 837 | 192 | 22 9 | 1036 | 424 | 40 2 | 1228 | 540 | 43 8 | 1040 | 281 | 26 9 |

A Total heat production (Cal/m²/d) derived from oxygen consumption (4 8 × ml $O_2$ = calories)

B Heat of vaporization of water (Cal/m²/d) derived from insensible water loss (0 6 × C $H_2O$ = calories)

C Heat of vaporization of water per cent of total

*Table 2 Influence of Saran Wrap Dressing on Insensible Water Loss and Metabolic Rate of the Burned Rat*

| DAY POST BURN | BURN COVERING | INSENSIBLE WATER LOSS Gms/M²/24Hr | CALORIES PER SQ METER/24 HR TOTAL | CALORIES PER SQ METER/24 HR VAPORIZATION WATER | WATER VAPORIZATION % OF TOTAL CALORIES |
|---|---|---|---|---|---|
| RAT D 15% BURN (PRE BURN) CONTROL | none | 380 | 989 | 228 | 22.3 |
| 13 | *none* | 746 | 1251 | 454 | 36.2 |
| 13 | Saran Wrap | 430 | 1083 | 258 | 23.9 |

*Table 3 Influence of Skin Excision of Metabolic Rate with Insensible Water Loss in the Rat*

| RAT | % SKIN EXCISION | RECTAL TEMPERATURE C | INSENSIBLE WATER LOSS Gm/M²/24Hr | CALORIES/SQ M/24 HR TOTAL | CALORIES/SQ M/24 HR VAPORIZATION WATER | WATER VAPORIZATION % OF TOTAL CALORIES |
|---|---|---|---|---|---|---|
| A Control | | 36.4 | 441 | 912 | 261 | 28.9 |
| 3 hrs post excision | 10% | 35.8 | 1482 | 1297 | 892 | 68.7 |
| Coverage with skin | | 36.2 | 465 | 940 | 279 | 29.7 |
| B Control | 10% | 36.1 | 414 | 981 | 248 | 25.3 |
| 3 hrs post excision | | 34.3 | 1354 | 1152 | 812 | 70.2 |
| Coverage with skin | | 36.0 | 409 | 954 | 245 | 25.7 |
| C Control | | 36.8 | 410 | 954 | 246 | 25.7 |
| 3 hrs post excision | 30% | 36.5 | 1251 | 1132 | 755 | 66.0 |
| 17 hrs post excision | | 34.0 | 1915 | 1386 | 1152 | 83.1 |
| 24 hrs post excision | | 32.1 | 1699 | 1180 | 1022 | 86.6 |
| 48 hrs post excision | | 32.0 | 1629 | 1111 | 974 | 87.6 |

quickly attained a rate five times normal and there remained until the animal died There was a concomitant rise of metabolic rate of 10 per cent above normal however the percentage of heat loss by vaporization of water increased progressively and just before death it represented 87 per cent of the total heat produced by the animal (Table 3, Rat C) The rectal temperature decreased gradually during the 18 hr period while the animal shivered and drank water copiously The water taken was equal to the water lost insensibly and the urinary flow was maintained

## DISCUSSION

Following a full thickness burn covering 5 to 20 per cent of the rat s body the rate of losing water through extrarenal channels is first decreased and then increased The period of decreased insensible loss of water lasts 1 to 2 days corresponding roughly to the shock period However, the animals were not anuric maintained normal postural attitudes and moved about their cages during this time consequently traumatic shock cannot be ascribed as the cause of the early depression of water loss

Neither is this early depression attributable to changes in body temperature the decline in cutaneous and rectal temperatures during this period were insignificant

A decline in metabolism of endocrinal origin is a possible cause of the primary evanescent depression of insensible water loss The turn over rate of $I^{131}$ within the thyroid gland of burned rats is depressed during this same time (Reichlin and Lieberman unpublished data)

The period of elevated rate of loss of water extrarenally consists of two parts the first lasting from the third to the fifth post burn day until full separation of the eschar (the eschar period) and the second beginning with the separation of the eschar and ending with complete healing of the wound (the open wound period) During the first part the rate of loss of water usually increases rapidly to a level where it remains fairly stable until the eschar separates then the rate suddenly increases and subsequently gradually falls as the wound closes During the period of elevated insensible water loss the metabolic rate is increased significantly However the rate of increase of oxygen consumption is generally calorically less than the increase of caloric loss incident to the increased rate of insensibly lost water This suggests that the increased metabolic rate during the escharotic and open wound periods is secondary or stimulated by the increased rate of heat loss attributable to the evaporation of water through and from the eschar and the open wound

In support of this view is the remarkably rapid drop of the metabolic rate (oxygen consumption) and evaporative loss of water to normal coincident with coverage of the open wound or eschar with a water impermeable dressing (Saran wrap) In addition the same order of decline in loss of water insensibly and metabolic rate follows the reapplication of the excised skin to a surgically denuded area Another observation adding support to this view is by lowering the environmental temperature to within the range of 2 C ± of the wound temperature the closer is the coincidence of the increments of metabolism and heat loss through the vaporization of water

These observations suggest that control of the environmental temperature and the construction of a dressing that will restrict but not prevent the trans it of water through a wound would reduce significantly the metabolic de

mands of a burned mammal and thereby permit an easier solution of the problem of post burn starvation.

The fact that a thermal injury remarkably changes the relationship existing between total heat loss and metabolic rate and the rate of loss of heat through the vaporization of water [heat loss by vaporization = 21 ± 2 per cent of total heat loss (normal)—heat loss by vaporization = 10 to 60 per cent of total heat loss (after larger burns)] obviates the use of the rate of insensible loss of water as a measure of the metabolic rate of burned mammals.

The faculty of a partially flayed animal to maintain life is in part dependent upon the animal's ability to increase catabolism (heat production) so as to meet the immutable increase in heat loss attendant upon the vaporization of water from the wound surface. The increased heat loss associated with a 10 per cent flaying can be met; that associated with a 30 per cent skinning usually cannot be with environmental temperatures less than 28° C.

Thermally killed skin, although hard and dry, is much more permeable to water than living skin. The rise in rate of water loss attending burning takes place through it, as attested by the immediate fall of insensible water loss to normal upon coverage of the eschar with Saran wrap.

The increased rate of vaporizational heat loss through burned skin and later from the open wound may be a most important factor in the genesis of the increased metabolism and the mortality of burned mammals.

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## A PLANNED EVALUATION OF EARLY EXCISION OF MORE THAN TWENTY-FIVE PER CENT OF THE BODY SURFACE IN BURNS*

BRUCE G. MACMILLAN AND CURTIS P. ARTZ

Although survival times have increased in extensively burned patients, modern advances in therapy have not appreciably decreased the mortality rate in this group of patients. Once the initial period of resuscitation has been successfully managed, patients with large areas of full thickness burns are faced with the deleterious effects of invasive infection until complete skin coverage has been accomplished. The magnitude of this threat is clearly defined when mortality figures in burned cases are analyzed. Eighty-two deaths have occurred in the 1,000 burned patients treated at the Surgical Research

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Unit Brooke Army Medical Center Fort Sam Houston Texas between the years 1950 and 1956 Of these 82 deaths 46 (or 56 per cent) died from uncontrolled invasive infection In the group of patients who died from septicemia the mean total body burn was 55 per cent the mean area of full thickness burn was 37 per cent and the mean burn index (third degree plus one half second degree) was 50

In view of the present refinements in supportive therapy and the continuing threat of infection a careful evaluation of early removal of extensive areas of full thickness burns was planned

METHOD

For this evaluation patients admitted since September 1 1955 with an expected mortality of 50 per cent were selected Individuals between the ages of 5 years and 55 years were studied Only patients with 25 per cent or more of their body involved in full thickness burns and with a burn index in the range of 40 to 60 were included Those patients admitted after the fifth post burn day were treated by conventional methods and those admitted prior to this time were excised Excision of the areas of full thickness burn down to fascia was performed in one or two stages between the second and the fifth post burn days Complete skin coverage was achieved either immediately or 2 days later by autografts and/or homografts For immediate post burn resuscitation whole blood (0 5 cc /kg /per cent burn) was used for colloid replacement and lactated Ringer s solution (1 5 cc./kg /per cent burn) was used for electrolyte replacement All patients were placed on penicillin and streptomycin during the first 5 post burn days Antibiotic therapy for septicemia was determined from bacterial sensitivity and administered in the following dosage every 24 hours cathomycin 1 0 gm bacitracin 300 000 units erythromycin 2 0 gm chloromycetin 3 0 gm and polymixin 200 mg All burned surfaces not excised were treated by the exposure method until healing or bacterial autolysis was noted Areas of full thickness burn in the conventional group were prepared for grafting by aggressive debridement and saline wet dressings

**Technical Considerations** An early accurate appraisal of full thickness burn involvement can best be determined from the resultant loss of sensation The inability to perceive pain usually signifies complete destruction of the skin however it must be borne in mind that some areas of the body are relatively insensitive such as the buttocks and the upper thighs In excising areas of full thickness burn it is better to err on the side of over excision since the remaining third degree burn introduces infection jeopardizes the graft take and delays wound closure

The depth to which areas of full thickness burn are excised likewise greatly influences primary wound healing Incomplete removal of underlying fat results in a high percentage of graft failure Since early complete wound closure is essential for the establishment of a protective barrier against bacterial invasion all areas have been excised down to the underlying superficial fascia With good hemostasis grafting can be successfully accomplished on this base however if bleeding cannot be meticulously controlled the incidence of graft failure rises precipitously Under these circumstances the wound is dressed over dry fine mesh gauze and grafting delayed for a period of 48 hours

Bleeding is minimal if the excision is performed between the first and the third post burn day After this period of time, bleeding is more profuse and its control more difficult To minimize the operative time all excisional procedures have been performed by utilizing 2 complete surgical teams The average total operative time has been 2 hours Cyclopropane anesthesia has been found to be the anesthetic agent of choice

For skin coverage after the excisional period, all of the patient's uninvolved skin has been utilized at the first grafting procedure Priority areas for coverage with autogenous skin are exposed fat tendons and joints Excised areas which could not be initially covered with autogenous skin have been covered with homografts Fresh cadaver homografts have resulted in the highest percentage of homograft takes Viability of homografts has ranged from 21 to 57 days When homografts have been rejected autogenous skin obtained from healed donor sites or from healed areas of second degree burn has been used to complete the skin coverage

RESULTS

Of the 80 patients admitted between September 1 1955 and September 1 1956 13 patients were included in this study Five cases were treated by early excision and 8 cases were treated by conventional methods The results obtained in the excisional group are shown in Table 1 In this group of patients the area of full thickness burn ranged from 30 per cent to 43 per cent and the area of total body burn varied from 12 per cent to 53 per cent The largest full thickness burned area excised at 1 operation was 32 per cent of the body surface The interval between burn and initial excision varied from 1 to 2 days excluding 1 patient who was excised on the fifth post burn day Four patients underwent 2 procedures and 1 patient 1 procedure to remove all full thickness burn involvement Blood lost during the excisional procedures ranged from 500 cc to 2 500 cc Three cases were grafted at the time of excision and 2 cases were grafted 48 hours after excision The lowest per cent take of autogenous skin grafts was 95 per cent and of homogenous skin grafts was 80 per cent Complete autogenous skin coverage was accomplished in 1 of the 5 patients between the 28th and the 37th post burn days None of these 4 patients developed septicemia One patient (L H , Table 1) died from a refractory pseudomonas septicemia on his 33rd post burn day before skin coverage could be accomplished In this case there was insufficient uninvolved skin available for coverage with autografts when the initial homografts were rejected Two of the 5 cases had uneventful recoveries Two of the 5 cases expired as the result of unrelated secondary complications One (H I Table 1) expired on his 30th post burn day after being given inadvertently an overdosage of morphine intravenously The other (R D Table 1) died suddenly on his 42nd post burn day from a probable pulmonary infarction

In the group of patients treated by conventional means the area of full thickness burns varied from 40 per cent to 48 per cent and the total burned areas varied from 40 per cent to 62 per cent Two of the patients in this group survived and the remaining 6 patients died Septicemia was the cause of death in all of the fatalities In those patients who were grafted the initial skin was applied on the 18th and the 30th post burn day respectively In the group of 6 patients dying from septicemia the initial positive blood

*Table I Summary of Experience of Early Removal of Extensive Areas of Full Thickness Burns in Five Cases*

| PATIENT SEX AGE | BURN EXTENT PER CENT TOTAL 3 | | BURN INDEX | SITE OF BURN | EXTENT OF EXCISION PER CENT | TIME DAYS BURN TO EXCISION | BLOOD REPLACEMENT DURING EXCISION | TIME DAYS EXCISION TO GRAFTING | GRAFT TAKE PER CENT | TIME DAYS BURN TO COMPLETE COVERAGE | COMPLICATIONS | TIME DAYS BURN TO DEATH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F H<br>F [illegible] | 4[illegible] | 30 | 37½ | Back<br>Buttocks<br>Posterior<br>Thighs | 30 | 1 | 1500 cc | 2 | Auto— 9[illegible]<br>Homo— 9[illegible] | 37 | None | |
| [illegible]<br>M 3[illegible] | 4[illegible] | 36 | 40½ | Ant chest<br>Abdomen &<br>Rt arm | 32 | 1 | 1500 cc | 2 | Auto— 9[illegible]<br>Homo— 9[illegible] | 30 | Decubiti<br>both heels | |
| | | | | Thighs | 4 | 13 | None | 3 | Auto—100 | 30 | | |
| F H<br>M 31 | [illegible] | 43 | 49 | Buttocks<br>Legs | 40 | 2 | 1000 cc | 0 | Homo— 80 | | Septicemia | 33 |
| | | | | Hands | 3 | 4 | 1000 cc | 0 | Auto— 8[illegible] | 11 | | |
| H F<br>M [illegible] | 12 | 30 | 37½ | Arms<br>Chest | 15 | 2 | 750 cc | 0 | Auto— 9[illegible]<br>Homo— 80 | 28 | Morphine<br>intoxication | 30 |
| | | | | Abdomen<br>Back | 1[illegible] | 4 | 500 cc | 0 | Auto— 9[illegible]<br>Homo— 80 | 28 | | |
| R D<br>M [illegible] | 13 | 31 | 37 | Legs | 13 | 5 | 1500 cc | 0 | Homo—100 | 3[illegible] | Probable<br>pulmonary<br>infarct | 42 |
| | | | | Chest<br>Rt Arm | 18 | 9 | 2,00 cc | 0 | Auto— 9[illegible] | 30 | | |

*Table 2. Summary of Experience in Eight Cases with Extensive Areas of Full Thickness Burns Treated by Conventional Methods*

| Patient Sex Age | Burn Extent Per Cent Total | Burn Extent Per Cent 3° | Burn Index | Site of Burn | Time Days Burn to Grafting | Time Days Burn to Contract | Time Days Burn to Septicemia | Organism and Sensitive Antibiotic* | Time Days Burn to Death |
|---|---|---|---|---|---|---|---|---|---|
| M.T. M 30 | 10 | 30 | [illegible] | Legs Buttocks Back | 32 | 90 | | | |
| V.I. F 7 | [illegible] | 19 | 11 | Back Abdomen Arms, legs | 21 | 135 | | | |
| M.C. M 21 | 55 | [illegible] | 15 | Face, arm Legs, chest Buttocks | 30 | | 26 | M. Pyogenes C B | 33 |
| S.N. F 6 | [illegible] | 36 | 11 | Arms, legs Abdomen | | | 7 | Pseudomonas Px Proteus (resistant) M. Pyogenes C B | 19 |
| R.H. M 32 | 57 | 30 | 11 | Arms, legs Abdomen Back | | | 11 | M. pyogenes B Pseudomonas C E | 24 |
| R.C. M 18 | 52 | 31 | 12 | Arms, legs Abdomen | 18 | | 17 | M. pyogenes B | 21 |
| R.O. M 9 | [illegible] | 29 | 12 | Arms, back Legs | | | 7 | Unknown | 11 |
| E.S. M 19 | 62 | 18 | 62 | Arms, legs Back, abdomen | | 63 | [illegible] | Pseudomonas A C M. Pyogenes B E Ca | 73 |

C—Chloromycetin A—Aureomycin B—Bacitracin T—Terramycin E—Erythromycin Ca—Cathomycin Px—Polymixin

cultures were obtained between the seventh and the 17th post burn days Although all patients with septicemia were treated with the appropriate specific antibiotic only 1 patient (E S Table 2) responded initially to specific antibiotic therapy On this patient s 51st post burn day, he developed a septic ileo-femoral thrombophlebitis which resulted in death on his 73rd post burn day The organism most frequently recovered from the blood stream was *Micrococcus pyogenes* coagulase positive In the group of *patients developing septicemia death occurred from the 11th to the 33rd* post burn day excluding the patient who developed septicemia late in his post burn course as the result of a septic ileo femoral thrombophlebitis Skin coverage in the 2 survivors was accomplished on the 90th and the 135th post burn days respectively

SUMMARY AND CONCLUSIONS

While the 5 patients treated to date by this aggressive attack on large areas of full thickness burns represent a small series certain observations can be made No deaths in this series can be attributed to operative intervention in the early post burn period Early excision and grafting of a full thickness burn wound involving 25 per cent or more of the total body surface can preclude invasive infection Antibiotic therapy alone cannot protect the extensively burned patient against the rigors of bacterial invasion The longer the interval between burn and excision the greater are the technical difficulties and the less effective the procedure Experience gained to date with early removal of extensive areas of full thickness burns justifies its further evaluation

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## PROTECTION AGAINST CONTACT AND FLASH BURNS*

GEORGE D ZUIDEMA NEVILLE P CLARKE JAMES R PRINE
AND EDWIN W SALZMAN

Each year many hundreds of man hours of productive effort are lost through one type of burn injury or another For example contact burns from the accidental ignition of clothing occur with alarming frequency A type of flash burn occurs in aircraft accidents or industrial explosions when liquid fuel explodes and burns At yet a third level atomic warfare has brought with it another type of flash burn that is of importance to military and civil defense personnel

Considerable effort has been exerted to study the characteristics of simulated atomic flash burns in order to understand the basic pathology and develop protective clothing for the armed forces or civil defense groups However comparatively little experimental effort has been directed toward

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such common problems as contact burns or the type of flash burn encountered in the explosion of liquid fuel

Colebrook[1] has studied this problem clinically on the burn service of a *large accident hospital His interest was based on the clinical impression* that the severity of injury following contact or flash burns varies widely and is to a large degree dependent on the clothing worn A breakdown of the fabrics worn by 92 patients admitted to his service with severe thermal injury is as follows

| | |
|---|---|
| Cotton | 61 |
| Viscose Rayon | 19 |
| Wool | 4 |
| Cotton and Wool | 3 |
| Acetate Rayon | 2 |
| Cotton and Viscose Rayon | 2 |
| Viscose and Acetate Rayon | 1 |

In his series all cellulose fibers were indicted with cottons by far the commonest offenders Of the 4 patients wearing woolen materials these people were without exception either epileptics or aged persons who had fallen directly into an open fire The absence of burns due to the newer synthetic fabrics can be attributed in part to their failure to support combustion but is also due to their comparatively infrequent use in 1951 at the time of the report

The present study represents an attempt to quantitate and establish an experimental approach to evaluating the burn protection offered by various fabrics Contact burns and flash burns simulating fuel explosions were selected for the study

## METHOD

Sprague Dawley rats weighing 250 to 350 gm were clipped with surgical shears to remove the hair from thorax and abdomen Twenty four hours later the rats were weighed and anesthetized with pentobarbital sodium 30 mg/kg The animals trunks were covered with bands of fabrics stapled together to assure a snug fit

In 1 series of 198 rats a standard wick of fabrics was prepared and heated to the ignition or melting point If the fabric supported combustion it burned down to the animal s body and produced a contact burn If it melted or failed to support combustion no burn resulted Each of 32 fabrics was tested on 6 separate animals in this way

The second series of 216 animals was prepared as above but each was held against an asbestos panel with a circular opening 2.5 cm in diameter The heat source a Meker burner using natural gas was applied to this opening and a temperature of 1204 C (2200°F) was delivered to each animal for 3 seconds Each of 33 fabrics was tested on 6 separate animals and 18 control animals wearing no fabrics were similarly exposed The results in each series were recorded in the same manner The burns were measured and the gross extent of each lesion was estimated at frequent regular intervals The clinical course of each burn was recorded and in addition representative animals from each group were photographed in color at 24 hours 7 15 and 22 days after burning Each animal was weighed weekly

during convalescence One typical member of each group of 6 rats was sacrificed at 24 hours and another at 15 to 18 days after exposure for microscopic study of the affected area The remainder of the animals were observed until completely healed and average healing times were determined for the various fabrics

RESULTS AND DISCUSSION

In general 3 parameters of measurement were available (1) average healing time (2) gross pathological grading and (3) microscopic pathological grading The gross results were tabulated as + ++ +++ on the basis of extent of lesions hemorrhagic areas at margin apparent depth and rate of sloughing This grading system proved to be especially useful in the flash burn series where each animal was exposed to a constant stimulus

Microscopic grades of severity were recorded as 1 to 4 as follows

(1) Superficial damage only characterized by local autolytic change thickening of the stratified squamous epithelium and occasional small increases in the interstitial spaces

(2) Coagulative necrosis of surface tissue extending to less than half the thickness of the corium Some cases in this group showed destruction of the stratified squamous epithelium and all showed coagulative necrosis of the superficial collagen bundles

(3) Coagulative necrosis extending the full thickness of the corium but not involving cutaneous muscle or subcutaneous tissue

(4) Severe coagulative necrosis involving full thickness of corium as well as subcutaneous fat and muscle

This type of classification is admittedly crude and depends on selecting a truly representative sample of burned tissue for examination The use of several sections from each block tends to minimize this possible error but cannot completely remove it The major changes seen in the sections taken at 15 days in comparison with those cut 24 hours after exposure are that the former show thickening of the epidermis and greater leucocytic infiltration This likely represents organization and secondary bacterial invasion of greater or lesser degree

Microscopically it was not possible to detect any significant morphological difference between burns of flash and non flash origin under these experimental conditions

There was no histological picture typical of any specific fabrics tested That is there was no microscopic evidence that a burn involving nylon for example differed in any specific way from any other fabric On early gross examination certain synthetic fabrics apparently melted into the skin when exposed to the Meker burner but this did not appear to increase the severity of the burn or lengthen healing time

For burns of equal depth that is equal histological grade non flash burns healed faster than flash burns thus undoubtedly reflecting the fact that the latter involved much greater total area This is also seen in an analysis of 20 grade 3 flash burns The mean healing times for the 3 gross classifications (+ to +++) then present a straight line relationship showing that within a given histological grade (grade 3) the less extensive gross burns (+ to ++) heal more rapidly than do the more severe burns (++ and ++ to +++) This supports the use of 3 parameters of measurement for extent

depth or healing time alone are not adequate to permit estimation of the other measures Given any 2 parameters the third could be predicted with fair accuracy within general limits

Utilizing these 3 parameters then in order of decreasing importance protection against this type of flash burn was found to lie in (1) weight or thickness of the material with heavy fabrics offering greatest protection (2) ability to conduct heat or char (a quality inherent in the material) (3) color with dark colors protecting less than light (4) flame retardent treatments

Under experimental contact burn conditions wool nylon, dacron and flame retardent treated cotton failed to ignite and no burn damage resulted Cotton synthetic combinations burned sluggishly, but flame retardent treatment prevented this damage Every sample of untreated cotton produced significant clinical burns

Loose fitting garments allowed free circulation of oxygen between fabric and skin The resulting burns were many times as severe as those seen with snug fitting samples of the same fabric

### CONCLUSIONS

This experimental study has emphasized the major qualities of fabrics which contribute to their ability to protect against thermal injury It has also pointed out the failure of certain materials to protect and offers experimental evidence which supports the major clinical survey of a similar kind

Colebrook[1] stated that in 1905 *The Lancet* stated that the days of flannelette should be speedily numbered because of its high flammability and the great number of accidents associated with its use Fifty-one years later flannelette remains in wide use and flame proofing does not appear to be economically feasible or at any rate is not generally accepted Furthermore we see no really active efforts to develop better substitutes or adequate flame retardent treatments It is hoped that clothing manufacturers will come to appreciate the importance of this step and furthermore exercise judicious choice of the newer synthetic fabrics and synthetic cellulose combinations

### SUMMARY

Criteria for histological grading of thermal injury have been presented In studies of flash burns and contact burns on rats grading based on these criteria have shown a positive correlation with gross pathological grading and with healing time These parameters have been employed in the estimation of the protective value of various fabrics

### REFERENCE

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# EFFECT OF HORMONES ON THE METABOLISM OF SURGICAL PATIENTS*

HELENA GELDER, EDWARD A. FREI, DONALD L. WEEKS, JR.
AND JOHN M. BEAL

The catabolic response to surgery has been shown to be reversible to a degree which depends upon the extent of trauma and the continuous administration of sufficient nitrogen, calories, and potassium to cover the needs during the postoperative period.[1] It became apparent that a part of the catabolism or of negative nitrogen balance which occurred postoperatively was due to the withdrawal of nitrogen from the intake during this period. It was suggested that in the constant dynamic events occurring in the breakdown and repair of tissue, a situation of stress does not necessarily delay or repress anabolism but may merely accelerate catabolism.

Testosterone has long been known to be anabolic and has been utilized in attempts to improve the nutritional state of certain patients.[2] The associated androgenic effect limited its usefulness. The development of a derivative of testosterone (17α-ethyl-17-hydroxy-19-nor-4-androsten-3-one), known as Nilevar® with anabolic properties and without significant androgenic effect initiated the present studies. The purpose of the work was to determine whether postoperative catabolism could be further reduced by administration of this hormone.

It has been shown in this laboratory that testosterone does not affect the nitrogen balance postoperatively if nutrients are not supplied at the same time. In the present report, 10 patients on a constant intake of nutrients throughout the operative period have been studied. Three patients were given Nilevar, 1 was given testosterone proprionate, and 6 were on a similar regime except that they received no hormone. A preliminary report on some of these patients, including case records, has been reported elsewhere.[3]

## METHODS

The 10 patients, 4 female and 6 male, in this study were placed on the metabolic ward of the Department of Surgery. The operative procedures were considered comparable in magnitude. In 9, a partial gastrectomy was performed and in 1 a resection of the sigmoid colon (patient M.M.). They were weighed daily and all intake and output were accurately measured. Oral feeding in 3 patients consisted of accurately measured regular diets, the food values for which were taken from standard diet tables. The other patients received a 6 feeding homogenized mixture of casein (Casec®) or milk, a fat emulsion (Ediol®), dextrose, and salts. The formula was designed to supply about 2000 calories and 0.2–0.3 gms. nitrogen per kilogram of body weight and samples were analyzed for nitrogen, fat, sodium, potassium, water and total mineral content. After operation, intravenous nutrients and

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Fig 1 Body weight balance data and caloric intake of control patient C B F

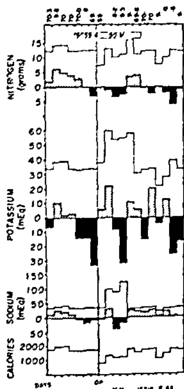



Fig 2 Body weight balance data and caloric intake of patient M M who received Nilevar

water were adjusted so as to maintain the preoperative level of nitrogen electrolytes and where possible calories until oral feedings were resumed Unfortunately, in several instances the caloric intake by intravenous feedings was lower Nilevar and testosterone proprionate were administered intramuscularly in doses of 25 or 50 mg daily beginning 1 to 3 days preoperatively Chemical methods for analysis of formula and excreta have been described elsewhere [3]

### RESULTS

Two typical experiments are shown in Figures 1 and 2 Body weight nitrogen potassium, and sodium balances and the caloric intake are plotted daily on a patient (M M ) who received Nilevar and on a control patient (C B F) who was on a similar regime but did not receive hormones The negative nitrogen balance is definitely less in the Nilevar patient than in the control and the potassium balance parallels the nitrogen balance

Table 1 is a summary of the results of weight and nitrogen balance changes on the 6 control patients 3 patients receiving Nilevar and the 1 patient receiving testosterone All the patients were on an essentially similar regime In all the cases except L F G R. and N A oral intake was very accurate because of the administration of the fluid formula described above as the sole oral feeding In spite of the great care in measurement of both intake and output in this study there were still sizeable daily fluctuations in nitrogen balance as note in the 2 examples in Figures 1 and 2 The days of study were therefore divided into periods of 3 to 6 days 1 period before operation and the other 2 after operation The results in the table give the weight change and the nitrogen balance in kilograms and grams respectively per day in each period

The previous physical status and dietary habits of the patient and ability

*Table 1 Weight Change and Nitrogen Balance in Surgical Patients with and without Hormones*

| PATIENT SEX | AGE YRS | WT kg | PREOP PERIOD DAYS IN PER | PREOP PERIOD HORMONE (d) | PREOP PERIOD WT Δ K/d | PREOP PERIOD N BAL gm/d | POSTOP PERIOD — 1 DAYS IN PER | POSTOP PERIOD — 1 HORMONE (d) | POSTOP PERIOD — 1 WT Δ K/d | POSTOP PERIOD — 1 N BAL gm/d | POSTOP PERIOD — 2 DAYS IN PER | POSTOP PERIOD — 2 HORMONE (d) | POSTOP PERIOD — 2 WT Δ K/d | POSTOP PERIOD — 2 N BAL gm/d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C B—M | 42 | 70 | [illegible] | — | −0.1 | −0.3 | 6 | — | −0.3 | −2.3 | 5 | — | 0 | +1.1 |
| G H—F | 42 | 52 | 2 | — | −0.1 | +0.8 | 4 | — | −0.5 | −10.1 | 5 | — | 0 | −1.0 |
| C L—M | 52 | [illegible] | 5 | — | −0.4 | −3.5 | 5 | — | 0 | −9.0 | [illegible] | — | −0.6 | [illegible] |
| G R—M | 63 | 70 | 4 | — | −0.1 | +2.8 | 4 | — | 0 | −0.2 | 4 | — | −0.2 | −2.6 |
| L F—M | 49 | 65 | 4 | — | −0.2 | [illegible] | 4 | — | −0.3 | −3.0 | 5 | — | −0.1 | −2 |
| C B—F | 37 | 57 | 2 | — | 0 | +2.1 | 5 | — | −0.1 | −4.2 | 4 | — | 0 | −3.9 |
| Av | | | | | −0.1 | +1.2 | | | −0.2 | −4.8 | | | −0.2 | −2.9 |
| N A—M | 50 | 67 | 6 | N 1d | 0 | +1.7 | 4 | N 4d | −0.4 | 0 | 3** | N 3d | −0.4 | −7.0 |
| M M—F | 66 | 70 | 3 | N 3d | −0.2 | −0.1 | 4 | N 4d | −0.3 | −1.9 | 4 | N 2d | +0.4 | +0.8 |
| I D—F | 26 | 54 | 1 | N 2d | +0.2 | +6.0 | 4 | N 3d | +0.2 | +3.5 | 4 | — | −0.4 | −1.0 |
| Av | | | | | 0 | +2.5 | | | −0.2 | +0.5 | | | −0.1 | −2.4 |
| C K—M | 42 | 54 | 2 | T 2d | −0.7 | +2.5 | 4 | T 4d | −0.4 | −0.2 | | | | |

Patient M M had a hemicolectomy All the others had gastrectomy Nilevar (N) and testosterone proprionate (T) were given in doses of 25 mg daily except in the case of M M who received daily doses of 50 mg Nilevar d = day

**This patient (N.A) had a febrile complication 4 days postoperatively

to adjust to the standard diet of approximately 2000 calories per day determined their preoperative nitrogen balances Only 1 patient (L F) had a significant retention of nitrogen preoperatively possibly due to depletion due to the illness The positive nitrogen balance in the other patients undoubtedly represented an adjustment to the diet One patient (C I ) was in a negative balance in the preoperative phase as well as following operation

Although the amount of trauma was comparable in the group of patients in the series an individual variation in the response to the surgical procedure was to be expected The average nitrogen balance in the first period after operation was —1 8 gms per day with a range from —0 2 to —10 1 gms per day in the 6 control patients The average of the 3 patients receiving the anabolic hormone was —0 8 with a range of +3 5 to —2 5 gms per day and the patient receiving testosterone had a nitrogen balance of —0 2 gms per day

*Variable findings also characterized the second postoperative period* There was considerable difference between individuals in the time that it took to recover from the catabolic response to the procedure One patient in the control group and 1 in the Nilevar group had reverted to a positive nitrogen balance by the end of 4 to 5 days whereas some of the other patients remained in markedly negative balance Furthermore in 1 case (N A) a pulmonary complication on the fourth postoperative day resulted in delay of recovery and a markedly increased negative nitrogen balance in the second postoperative period

On inspection of the average nitrogen balance in Table I the anabolic properties of Nilevar appear to be effective in the postoperative period This effect exceeded the reduced negative nitrogen balance which occurred in the control patients in whom calories and nitrogen intake was maintained throughout the early postoperative period with intravenous infusions Testosterone had a similar but less potent effect in the single case studied However, the series is admittedly too short to give reliable statistical evidence of any difference between the effect of the 2 hormones or for that matter, to be definitive as to the effect of testosterone

The administration of hormones to these patients did not appear to affect significantly the change of body weight following operation which may already have been minimized by the high nutrient intake in the postoperative period

Although testosterone is known to cause salt and water retention the dose of Nilevar and in the 1 case testosterone given for the limited number of days in the present experiments resulted in no demonstrable sodium or water retention

DISCUSSION

The administration of a new anabolic hormone Nilevar, in conjunction with a high caloric and nitrogen intake postoperatively has been shown to decrease the negative nitrogen balance due to operative stress Testosterone proprionate in the same doses seems to have a similar effect It is probable that the mechanism of action of the 2 drugs is alike but this is yet to be proved

In this study patients undergoing comparable operative stress were deliberately chosen to facilitate analysis of the results It is not implied that

patients who are subjected to gastrectomy should receive such hormone therapy *without more extensive study of the problem. It is more likely that* patients in poor nutritive condition who must undergo severely traumatic surgery would benefit by a regime which would reduce the catabolism of surgery. Whether the hormones and a maintained high caloric and nitrogen intake will improve the postoperative period for such patients is still to be determined.

Preliminary RQ measurements[3] and data from calculations of the components of weight change have shown that the anabolism due to Nilevar occurs at the expense of fat which is apparently mobilized during the administration of the hormone. Whether this is a direct effect on fat or a secondary result due to the requirement of a source of energy for the synthesis of protein is yet to be determined. If the latter is the case, a high caloric source in the form of carbohydrate should spare fat during the administration of the hormone. The importance of maintaining caloric intake postoperatively has already been demonstrated in the previous work.[1]

Theoretically, it would seem that the prevention of protein breakdown should be beneficial in patients undergoing surgery. However, it will take a much longer series of patients to prove that the regime described above hastens recovery or reduces postoperative complications.

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## THE INFLUENCE OF GROWTH HORMONE ON PROTEIN METABOLISM*

MELVIN S. SCHWARTZ AND JOHN F. PRUDDEN

Protein metabolism in the normal person and in both the acute and convalescent burn patient has been studied with the aid of $S^{35}$ labeled methionine given intravenously in 100 microcurie doses. Sulfur labeled methionine was chosen since *prior evidence had indicated that this amino acid plays an important role in wound healing processes*[1] *in addition, the use of a labeled amino acid allowed for the synthesis in vivo* of tracer protein native to the body.

The incorporation of isotopic sulfur into the globulin and albumin fractions of the plasma as well as the plasma levels of the non-protein radio-sulfur were followed for an average period of 10 days after administration

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of the isotope In certain of the normal and patient studies 50 mg of growth hormone* was injected intramuscularly beginning at 1 to 4 days following the methionine and continuing at 6 hr intervals until a total of 1000 mg had been given Fifteen studies based on 6 patients and 2 normal individuals have been completed to the present time

Blood samples are collected without anticoagulants, primarily to avoid introducing carrier sulfur impurities such as those found with heparin This is critical since the analytical methods must necessarily be extremely sensitive to reflect accurately the low plasma levels encountered for the non protein sulfur and even for $S^{35}$ albumin and $S^{35}$ globulin late in the study The method used is essentially that of Tarver[2] modified by Gray and Seraille Involved are methanol precipitation of globulin trichloracetic acid precipitation of albumin and non protein sulfur precipitation by benzidine on the residual supernatant solutions from the latter two procedures Final radioactive measurements have been made through $\beta$ counting in a gas flow chamber

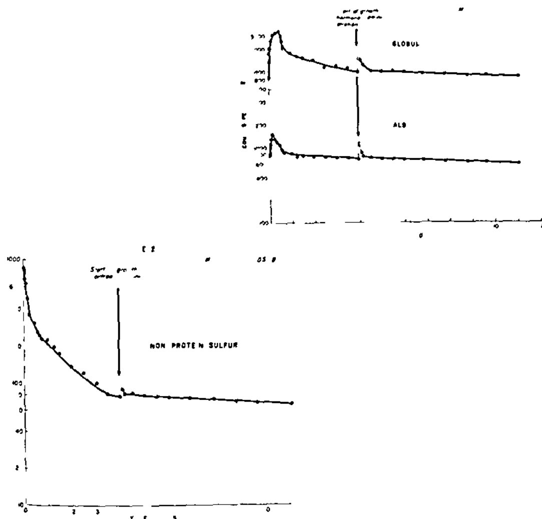
The curves representing the incorporation of radiosulfur into plasma albumin and globulin and the decline of non protein radiosulfur in the plasma are complex (See Figs 1 and 2) The peaks of the protein curves occur within the first 12 hours after injection of radioactive methionine at the end of 24 to 36 hours the same semilogarithmic graph exhibits a tendency to fall away in almost linear fashion Hypotheses about the metabolic pathways entered upon by methionine have been formulated in detail It is reasonable to say that with or without growth hormone administration each protein curve is the resultant of at least two simultaneous processes—that of the incorporation of methionine into the protein (the *synthetic* phase) and that of the release of radiosulfur during the course of physiological turnover (the *degradation* phase) However the reincorporation of $S^{35}$, already synthesized and degraded once, into a second metabolic cycle—reutilization—complicates the analysis of these graphical results

One simplification is possible It is reasonable to assume that the tail end of such curves (the linear portion found beginning with the fourth or fifth day after injection) represents essentially but one of these two main processes that of degradation On this assumption the half lives of the degradation phase have been calculated from the straight die away portions of the graphs Table 1 lists the typical values found for globulin catabolism

*Table 1*

| PATIENT | | TIME OF DETERMINATION POST BURN | HALF LIFE OF GLOBULIN CATABOLISM |
|---|---|---|---|
| AW | (with G H) | 1 month post burn | 3 4 days |
| | (with G H) | 2 months post burn | 9 7 days |
| | | 4 months post burn | 12 5 days |
| FM | | 2 months post burn | 6 0 days |
| | (with G H) | 4 months post burn | 15 0 days |
| RT | | *normal* | *14 0 days* |

*Growth hormone was supplied by The Armour Laboratories as Lot #R491025 it was free of vasopressor substances but was reported to contain 0 32 ± 0 098 units of thyrotropin per milligram




It has been a reproducible finding that as convalescence from burn injury proceeds the half life for release of $S^{35}$ from globulin lengthens Degradation—and possibly true globulin turnover itself if the convalescent is approaching a steady state—slows down and approaches in absolute value that of the normal individual It may be noted from Table I that the administration of growth hormone does not add any qualitative change to this general trend Globulin has been used to illustrate the phenomenon the albumin fraction of the plasma while also showing catabolic half time changes with convalescence behaves in a more complex fashion and its data are still undergoing study

Growth hormone administration while not grossly influencing the catabolic phase of these protein curves does have a pronounced effect almost immediately post injection and on all of the substances followed—i e albumin globulin and non protein sulfur (See Figs 1 and 2) Within 1 to 1 hours following the first administration of the hormone a pronounced rise or jump occurs in each curve studied For albumin and globulin these jumps are evidence of sudden sharp increases in the rates and degress of $S^{35}$ incorporation into the proteins which may be interpreted as distinct anabolic boosts These striking discontinuities in the graphs have been found in every instance of concomitant $S^{35}$ and somatotrophic hormone administration

*The unique picture thus presented allows for 3 main possibilities (1)* The rates of anabolism of albumin and globulin undergo absolute increases immediately after injection of growth hormone However the presence of the same type of discontinuity in the curve of non protein sulfur (see Fig 2) suggests not *absolute* anabolic boosts but rather (2) a shift in the chemical equilibria involved to increase the magnitude of the amino acid pool within the body, thus increasing the amino acid concentration in the plasma and in turn the synthesis of protein or alternatively, (3) alternatively a release of sulfur labeled amino acid alone or a release of both labeled amino acid and labeled protein into the plasma from storage in some organ or metabolic depot within the body, immediately after administration of growth hormone

Liver perfusion studies have lent some confirmation to the latter two hypotheses The speed with which the phenomenon follows upon hormone administration tends to favor the depot hypothesis and there is positive evidence that the isolated perfused liver can release plasma protein upon injection of growth hormone[3]

It would seem from these preliminary results therefore, that growth hormone has at least a short term positive anabolic effect, the net result of which is to boost protein synthesis This may result directly from increase of rates of anabolism but more probably occurs as a result of rapid mobilization from metabolic depots of stored protein and amino acid material Such action produces a sudden break in the curves of sulfur incorporation into albumin and globulin similar to that which would occur with a second injection of non protein $S^{35}$ into the circulation Indeed the curve of non protein sulfur shows evidence of such a reinjection This phenomenon associated with growth hormone is receiving further study in our laboratory at the present time

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# AN IMPROVED METHOD OF MEASURING LOW PLASMA HEMOGLOBIN LEVELS AND SOME PRELIMINARY CLINICAL CONSIDERATIONS*

J. V. Cockrell and H. N. Naumann

Interest in serum hemoglobin levels among surgeons has been mostly limited to conditions in which quite high levels occur, such as with pump-oxygenators, hemolytic transfusion reactions or in lower nephron nephrosis due to hemoglobinemia. These are usually measured by Ham's method.[1]

This investigation has been directed to develop a method of measurement and to study disease states with low abnormal plasma hemoglobin values, i.e., between 2.5 and 10 *mg. per cent*.

## METHOD

**Inhibitory Substance.** As shown by Creditor[2] and confirmed by Crosby and Furth,[3] substances exist in plasma which inhibit the accurate measurement of hemoglobin by the quantitative benzidine reaction of Wu.[4] These substances pose a special problem in dealing with low values, as in our study.

It was found by one of us (H. N. N.) that in preparing the standard and blank the influences of these substances could be compensated for by adding the unknown plasma after prior "deactivation" of its hemoglobin by $H_2O_2$. Thus unknown, standard and the blank are all subject to the same interference.

**Other Important Factors.** Hemolytic artefacts giving false high values of plasma hemoglobin may be caused by one or more of the following: prolonged application of venous tourniquet, skin antiseptic solution contaminating the needle, a small bore needle, water in the syringe, a poor venipuncture (important), roughness in handling the blood, improper anticoagulant, too rapid centrifugation, the very slightest agitation of the red cell mass during pipetting (important), and the slightest contamination of the glassware (important). Only benzidine base and not the dihydrochloride is suitable as reagent.

**Venipuncture and Plasma Separation.** A commercial heparin sodium solution, 0.2 ml. containing 1,000 U.S.P. units per ml., was measured under sterile conditions into a 20 ml. syringe. Skin antiseptic was applied and the skin was dried thoroughly before venipuncture. The tourniquet was not applied until immediately before venipuncture. Any difficulty in entering the vein produced artefactitious hemolysis and free flow of blood into the syringe was essential. Eleven milliliters of blood were drawn gently into the syringe and mixed with the heparin. Ten milliliters of the blood were then slowly expelled along the wall of a graduated centrifuge tube which was centrifuged at 1,000 rpm for 10 minutes. The supernatant plasma was aspirated by means of a pipette with attached rubber tubing without disturbing

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The authors wish to thank [illegible] Naumann for her library research and William Correll and Myra Lowery for their technical assistance.

the packed cells in the least was then recentrifuged and pipetted off again leaving about 0 5 ml in the tube

**Quantitative Benzidine Method** *Principle* The sensitivity of the method was increased by the highest possible concentration in the reaction mixture of the most concentrated reagents and least diluted plasma Recovery of hemoglobin was achieved by adding to the standard and blank solutions the same unknown plasma whose hemoglobin was deactivated by first adding $H_2O_2$

*Benzidine reagent* Ten grams of benzidine base (Harleco) were dissolved in 100 ml of glacial acetic acid at room temperature with occasional stirring After centrifuging the reagent was clear yellow and was used for one day only

*Hydrogen peroxide* Three per cent USP hydrogen peroxide was used undiluted and stored in the refrigerator

*Standard hemoglobin solutions* A normal blood sample the hemoglobin content of which had been determined by the iron method (Sunderman *et al*[5]) was diluted 1 1000 with distilled water to give a stock solution of about 15 mg per cent which was stable for 3 weeks in the refrigerator Working standards of 1 2 3 and 4 mg per cent were made fresh daily by appropriate dilution of small amounts of the stock standard with distilled water The original blood could be frozen in quantities of 2 ml in test tubes and was stable for long periods

*Twenty per cent acetic acid* Two hundred ml of acetic acid were diluted to 1 000 ml with distilled water

*Procedure* All glassware including syringes and needles were washed with 1 3 diluted concentrated $H_2SO_4$ free of dichromate followed by 5 rinses with tap water 3 rinses with distilled water and oven drying (avoiding rust from wire baskets)

One ml of plasma (if grossly hemolyzed and appearing brown to reddish brown prior dilution of 1 10 or more was necessary) obtained as described above was mixed with 1 ml of distilled water This plasma dilution in amounts of 0 25 ml was pipetted into 5 test tubes labelled B (blank) 1 2 3 and 4 (mg per cent hemoglobin standard solutions) and 0 25 ml of $H_2O_2$ was added to each tube and mixed While these mixtures were kept at room temperature for 10 minutes ( deactivation of hemoglobin ) another set of 5 tubes were labelled B 1 2 3 and 4 and in this order 0 5 ml each of water 1 2 3 and 4 mg per cent hemoglobin standard solutions diluted 1 2 were pipetted into the respective tubes 0 5 ml of benzidine reagent was added to each of the 5 tubes and mixed (Note One set of standards must be run with each unknown plasma ) One tube labelled U (unknown) contained 0 25 ml of plasma dilution 0 25 ml of water and 0 25 ml of benzidine reagent Then 0 5 ml each of the benzidine mixtures B 1 2 3 and 4 were pipetted into the corresponding tubes containing the deactivated plasma mixtures and 0 25 ml of $H_2O_2$ were added to the U tube with the unknown plasma All solutions were carefully mixed with any drops adhering to the wall of the tubes After 15 minutes 10 ml of 20 per cent acetic acid were added to each tube The purple colored solutions were mixed and the optical density (logarithmic numerals) was read in a spectrophotometer at a wave length of 500 mμ using the blank to zero the instrument We have used to advantage a Spectronic 20 (Bausch & Lomb) equipped with a Roto-Cell (A H Thomas)

*Table 1 Scheme of Improved Benzidine Method*

| | Plasma 1 2 | $H_2O_2$ | $H_2O$ | | Standard Benzidine Mixtures: Standards | Standard Benzidine Mixtures: Benzidine | $H_2O_2$ | | 20% Acetic Acid |
|---|---|---|---|---|---|---|---|---|---|
| Unknown | 0 25 | — | 0 25 | | — | 0 25 | 0 25 | 15 minutes reaction time | 10 0 |
| Standards 1 mg% | 0 25 | 0 25 | — | ← 10 minutes; 0 5 ml of each | 0 5 of 1 mg% 1 2 | 0 5 | — | | 10 0 |
| 2 mg% | 0 25 | 0 25 | — | ← | 0 5 of 2 mg% 1 2 | 0 5 | — | | 10 0 |
| 3 mg% | 0 25 | 0 25 | — | ← | 0 5 of 3 mg% 1 2 | 0 5 | — | | 10 0 |
| 4 mg% | 0 25 | 0 25 | — | ← | 0 5 of 4 mg% 1 2 | 0 5 | — | | 10 0 |
| Blank | 0 25 | 0 25 | — | ← | 0 5 of $H_2O$ | 0 5 | — | | 10 0 |

*Calculation* The optical densities of the 4 hemoglobin standards were plotted on standard graph paper against the concentration in mg per cent A graph was drawn and the value of the unknown read from the graph Usually the graph was almost a straight line and the calculation was made by interpolation This figure in mg per cent of plasma hemoglobin was corrected for the heparin dilution for variations in hematocrit adjusted for extra dilutions if any of plasma or for the final color solution

## DISCUSSION

*Normal values* By the use of the described method plasma hemoglobin values in normal individuals were found to range from 0 3 to 2 5 mg per cent with an average of 1 3 mg per cent

*Abnormal values* Preliminary studies have shown the following abnormalities

*Blood transfusion without operation* Bell[6] in our University Hematology Laboratory using Ham s method reported elevations of plasma hemoglobin are quite common following transfusion Our series in this field was small and showed minimal rises if any Probably degrees of compatability and age of the blood are important determining factors

*Table 2 Plasma Hemoglobin Levels After 500 cc Transfusion Without Operation*

| Indication for Transfusion | Before Transfusion | Immediately Following Transfusion | 1st Day Following Transfusion |
|---|---|---|---|
| Secondary Anemia | 1 1 Mg % | 1 9 Mg % | 1 0 Mg % |
| Secondary Anemia | 1 4 | 2 2 | 0 8 |
| Secondary Anemia | 0 6 | 1 1 | 0 5 |
| Secondary Anemia | 0 | 1 1 | 0 7 |

*Operation without blood transfusion* Few operations of any magnitude are done these days without accompanying transfusion The following were selected cases studied in which transfusion was either not performed or with held until after the post operative specimen was drawn They show definite but small degrees of hemoglobin elevation in two cases

*Table 3 Plasma Hemoglobin Levels in Operations Without Blood Transfusion*

| OPERATION | PRE OP | IMMED P O | 1ST P O DAY |
|---|---|---|---|
| Inguinal Herniorrhaphy | 1.9 Mg % | 0.6 Mg % | 1.4 Mg % |
| Inguinal Herniorrhaphy | 1.9 | 1.0 | |
| Ventral Herniorrhaphy | 1.1 | 1.3 | 1.4 |
| Amputation of Toe Under Tourniquet (700 mm for 20 min) | 1.5 | 1.9 | 1.1 |
| Ligation of Inf Vena Cava | 1.0 | 3.1 | 1.3 |
| Open Reduction of Fracture of Femur with Intra medullary Nail Fixation | 2.1 | [illegible] | 1.6 |

*Operation with blood transfusion* Shinowara[1] first reported studies on a radical mastectomy receiving transfusion Our studies also showed frequent rise above normal of values in patients undergoing major surgery requiring blood transfusions

*Table 4 Plasma Hemoglobin Levels in Operations with Blood Transfusions*

| OPERATION | CC OF BLOOD GIVEN | PRE OP | IMMED P O | 1ST P O DAY | 2ND P O DAY |
|---|---|---|---|---|---|
| Suprapubic Prostatectomy | 1,000 | 0.8 Mg % | 3.1 Mg % | 1.2 Mg % | |
| Extrapleural Thoracotomy with Plombage | 2,000 | 1.9 | 12.6 | 2.7 | |
| Subtotal Gastrectomy | 1,500 | 2.3 | 19.4 | 11.0 | 1.4 |
| Craniotomy with Resection of Temporal Lobe | 4,000 | 1.6 | 3.2 | 2.0 | 1.7 |
| Transurethral Resection of Prostate | 1,500 | 1.2 | 4.0 | | 2.3 |
| Mitral Commissurotomy | 1,000 | 2.9 | 38.[illegible] | 2.5 | |
| Suprapubic Prostatectomy | 500 | 0.4 | 9.1 | 1.4 | 1.4 |

*Drug influence* No elevations were demonstrated after either inhalation or intravenous anesthesia nor after administration of the common intra venous fluids

*Disease states* Abnormal levels (between 2.5 and 10 mg per cent) were demonstrated in patients with acute pancreatitis intra abdominal abscess

sickle cell anemia in remission, polycythemia vera, pseudomonas meningitis and in some patients with jaundice.

SUMMARY

1. An improved method for measuring low plasma hemoglobin levels is described based on a new principle to achieve maximum hemoglobin recovery. Necessary precautions are presented to avoid hemolytic artefacts.

2. Beginning clinical studies are reported. 2.5 mg. per cent has been found to be the upper limit of normal with our method. Elevated plasma hemoglobin levels have been demonstrated following operations with and without transfusion and in some disease states.

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## INTRA AND EXTRACELLULAR CHANGES OF FLUID AND ELECTROLYTES IN EXPERIMENTAL UREMIA*

B. F. RUSH, JR. AND H. T. RANDALL

This study was designed to obtain a more complete evaluation of the shifts of fluid and electrolytes that occur in acute renal failure. Three general methods were employed: 1) measurements of extracellular fluid and electrolytes, 2) measurements of intracellular fluid and electrolytes in muscle biopsies, and 3) balance studies.

### METHOD

Extracellular electrolytes were measured by standard methods previously described.[1] Plasma volume was measured by the use of Evans Blue dye (T-1824). Extracellular fluid volume was estimated by the diffusion of inulin and in 2 cases sucrose. Fluid spaces in the muscle biopsies were determined

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by the method of Mokotoff *et al* [2] using inulin in 5 cases and sucrose in 2 It is realized that the space defined by inulin or sucrose is at best, an approximation of true extracellular space When we speak of extracellular space in this article it must be understood that we are actually referring to that compartment measured by inulin or sucrose

Seven healthy mongrel dogs kept in an air conditioned kennel were used in these experiments Control studies on each dog were first done These consisted of a determination of the dog s weight plasma volume inulin or sucrose space plasma electrolytes and muscule biopsies to measure the muscle extracellular and intracellular fluid and electrolytes Following the control study acute anuria was created in the animals by tying off the ureters at the brim of the pelvis transabdominally, under general anesthesia The dogs were placed in metabolic cages and daily determination of plasma electrolytes weight intake and output of water and in some cases electrolyte balance studies were done The animals were maintained on nothing by mouth and it was planned to replace fluids intravenously in an amount approximately equal to 50 per cent of the daily weight loss, usually with a solution of 5 per cent dextrose and water Sodium and chloride ions lost via the gastrointestinal tract through vomiting were also replaced The volume of replacement varied more widely than planned but was always less than the daily weight loss

After 72 hours of anuria all of the previous control studies were repeated Determinations of the fluid volume and electrolyte content of the dead space in the renal pelvis and ureters in three animals revealed that loss of inulin or sucrose and electrolyte was not great enough to affect the study significantly

RESULTS

On the regime of fluid replacement described above the dogs lost an average total of 7 per cent of their control weight (range 1 to 10 per cent) at the end of 3 days of anuria Water balance studies revealed an average insensible loss of approximately 30 gm /kg /d

In order to present the greatest amount of data in the most concise form

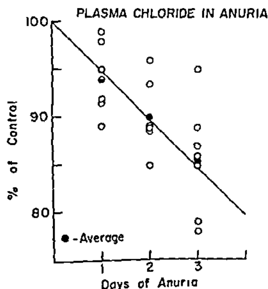



Fig 1 Change in plasma chloride in the course of 72 hours of anuria there is a relatively consistent decrease each day in the plasma concentration The average daily decrease can be plotted on an almost straight line When one considers that the extracellular volume is also falling over this period it is evident that the total loss of chloride from the extracellular space is considerable

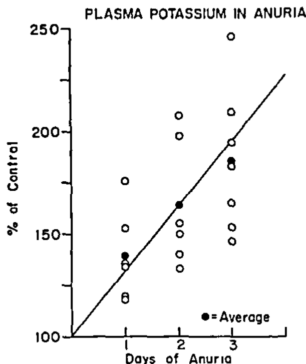



Fig 2 Changes in plasma potassium concentration in the course of 72 hours of anuria The rise in plasma potassium in the course of the regimen described was quite consistent with a straight line rise to a 100% increase in the 3 days Since the total potassium in the extracellular space is relatively small it does not require the addition of much more potassium to accomplish this change The consistent rise of potassium provides an interesting contrast to the changes seen in clinical material where this change is often variable

the following charts are arranged in the form of scattergrams in terms of a percentage of the control value the control value being set at 100 per cent Figures 1 and 2 show the remarkably consistent changes of plasma concentration of potassium and chloride Sodium (Fig 3) was somewhat variable with no significant average change Average chloride concentration decreased 15 per cent The changes recorded in extra and intracellular water and intracellular potassium and chloride are shown in Figure 4 and the significant features are pointed out by the legend The most interesting correlation made here is that despite the fall in chloride concentration of the plasma the extracellular space as indicated by 3 separate methods decreased in volume Intracellular chloride ion could not be presented adequately in this chart since the percentage increase was greater than the scale used Control

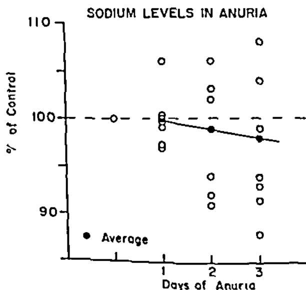



Fig 3 Changes in plasma sodium concentration in the course of 72 hours of anuria Changes in plasma sodium were quite variable and the average concentration of sodium did not deviate from normal over the period of anuria

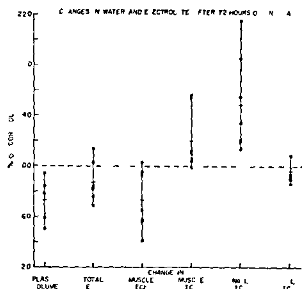


Fig. 1 Changes in intra and extra cellular water and extracellular sodium and chloride Extracellular water was decreased both in terms of plasma volume total extracellular water and extracellular water in muscle biopsies On the other hand intracellular water in terms of the muscle biopsy findings increased an average of 20% At the same time intracellular sodium increased and intracellular potassium decreased

values in 5 dogs showed an intracellular content of chloride ranging from 7 to 0 mEq/L of muscle water In 6 dogs after 3 days of anuria the intracellular chloride in muscle varied from 11 to 33 mEq/L of muscle water

Chloride balance studies in 3 dogs showed no evidence of extrinsic loss which would explain the observed drop in plasma concentration (Table I)

*Table I*

| DOG NO | CHLORIDE IN | CHLORIDE OUT | NET GAIN OR LOSS | OBSERVED LOSS IN ECF | UNACCOUNTED LOSS IN ECF |
|---|---|---|---|---|---|
| 112 | 41 0 | 56 6 | —15 6 | —149 | —165 |
| 139 | 97.2 | 118.2 | —21 0 | —51 | —30 |
| 113 | 61 7 | 12 1 | +49 6 | —123 | —173 |

Table I Chloride balance studies Studies in these 3 dogs showed that the net extracorporal loss of chloride could not account for the total amount of chloride lost from the extracellular fluid

DISCUSSION

The findings indicate that after 3 days of anuria on the regimen described there is a loss of water, sodium and chloride from the extracellular space Somewhat similar changes have been reported by others[3 4 5] This loss can not be fully explained on the basis of loss from the body At the same time there is an increase of water sodium and chloride in the intracellular space in muscle Intracellular potassium decreases and extracellular potassium increases These findings suggest that extracellular fluid and ions have shifted into the cells with a complementary shift of intracellular potassium and perhaps other ions out of the cell There are many possible explanations for the changes observed and we will not deal with them here On correlating the water balance data with the figures on intracellular water it is apparent that there has been no change or even an increase in total body water This has occurred despite the loss of weight of the animals Calculations of the water released from the tissues by the catabolism of fat and protein in the dogs over this period reveals that there is an adequate source for this water

Some have maintained that inulin shifts into the cells in the course of uremia[6] If this were the case here the same apparent changes noted in

studies of potassium sodium and chloride in the muscle would be found, but at the same time there would be an apparent increase in extracellular fluid both in the total body studies and in the muscle biopsies Also, the intracellular fluid in the muscle biopsies should appear to decrease—exactly the opposite of what was actually observed

In these studies the dogs were given fluids sparingly in accord with present concepts of the treatment of acute renal failure The changes found were however not at all characteristic of what one would expect in acute dehydration

Some clinical and experimental studies of the fluid and ion changes in uremia have indicated an increase in extracellular water A number of these studies have been based on calculations of the chloride space as an index of the extracellular space[7] If chloride shifts into cells during anuria such calculations would be most misleading

## SUMMARY AND CONCLUSIONS

Experimental renal failure was produced in 7 dogs by bilateral ureteral ligation Prior to ligation observations of the weight extracellular fluid and electrolytes and intracellular fluid and electrolytes of the dogs were done During 3 days of anuria daily observations of plasma electrolytes water balance weight and in some cases chloride balance were done At the end of 3 days of anuria the control studies were repeated Results suggest that in the course of anuria there occurs an extracellular dehydration with a loss of fluid from the extracellular compartment This change is accompanied by an increase in the cells of the extracellular ions sodium and chloride while some potassium appears to leave the cells

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# EFFICIENCY OF UTILIZATION OF ORAL FOODSTUFFS STUDIED BY CARBON DIOXIDE PRODUCTION*

JOHN M KINNEY WILLIAM G HAMMOND W BANKS ANDERSON AND ELLIOTT V MILLER

With the growing recognition of the key role of available energy in modern surgery a wide variety of feeding mixtures and parenteral fluids is being marketed—with special emphasis on the provision of more and more calories per unit volume This situation raises the need for a better understanding of the relationship between the rate of caloric supply and calorie utilization Efficiency of foodstuff utilization can be approached by measuring new tissue laid down (using body composition studies balance techniques etc.) The alternative approach is to measure the amount of food not laid down as *tissue and therefore undergoing combustion to yield a corresponding* amount of heat along with the necessary utilization of oxygen and production of carbon dioxide

A long term study of the factors influencing carbon dioxide production in surgical patients has been undertaken in this laboratory One of these factors is the increased production associated with the ingestion of food termed specific dynamic action or SDA The exact nature of the stimulus to this increased heat production or carbon dioxide output remains uncertain but there is reasonable agreement on certain facts

SDA is a mild heat stress not associated with digestive processes and is unavailable as energy for muscle work Some investigators have felt SDA to be a constant proportion of consumed calories (approximately 2 per cent for fat 5 per cent for carbohydrate and 10 to 30 per cent for various amino acids) However there is evidence that SDA may not be a biologic constant for a given food but rather may exhibit some variation depending on the condition of the patient and how the food is administered [1] The energy loss due to SDA varies from 5 to 15 per cent on an average mixed diet and is insignificant in active healthy men However this loss may assume added significance in depleted bed ridden patients where tissues function less efficiently and positive caloric balance is difficult to maintain

People probably differ in the hereditary control which they maintain over excess calories namely whether the food is burned in the metabolic furnace or stored as carcass fat Strains of mice have been shown to differ sharply in this oxidative tendency [2] This would appear to have its clinical counterpart in the constitutionally slender people who somehow refuse to gain weight despite caloric intakes above their metabolic demands People with endogenous obesity have been reported to have a reduced SDA,[3] indicating a tendency to store rather than burn extra calories

In brief SDA appears to be the sum of all the intracellular handling charges associated with moving food products along various intermediary

*From the Department of Surgery Harvard Medical School and the Peter Bent Brigham Hospital We wish to acknowledge the support for these investigations from the U.S Public Health Service

We wish to express appreciation to Dr F D Moore for his advice and encouragement to Mrs Ruth Daniels for laboratory assistance and to Miss Hewa Schieve for diet management

pathways some of which are associated with greater metabolic expense than others

There is little direct information regarding the effect of rate of administration of foodstuffs on SDA There is some evidence that the larger the meal (which might represent a faster rate of administration) the larger the loss due to SDA Lusk proposed that a plethora of digestion products in the tissues stimulated the heat increment of SDA[4] and recent studies have offered support for this The concept raises the possibility that slow rates of caloric administration might avoid most of the plethora and possibly lessen traffic along certain intermediary pathways which handle the overflow of digestion products and are associated with the extra carbon dioxide production To explore this possibility and to obtain further information about patterns of carbon dioxide production in patients receiving special nutritional mixtures the following introductory study was undertaken

METHOD

The studies presented here were performed with 6 normal subjects hospitalized on a surgical ward for a total of 40 days with measurements of carbon dioxide production being made at intervals of 45 minutes to 2 hours throughout each 24 hour period The food was carefully standardized by a research dietitian to provide 47 per cent carbohydrate 16 per cent protein and 36 per cent fat and was given in amounts of 1 600 cal 2 400 cal, or 3 200 cal Tube feedings were made by homogenizing and suspending the cooked food in a standard volume of water (usually 1 cc for each calorie) Tube feedings were given at uniform rates using a Bowman constant infusion pump connected to a plastic nasogastric tube The carbon dioxide measurements were 7 minute collections performed as previously described[6] Analyses for residual carbon dioxide in the apparatus were performed with a Haldane instrument Subjects had minimal physical activity during the study and were completely quiet in the supine position for 15 minutes or more before each run Data were calculated as carbon dioxide production in cc/m$^2$/min and the mixed diet as cal /m$^2$/hr

*Clinical Studies* The carbon dioxide production of 4 subjects during a 24 hour fast and during the initial fasting hours of other studies provided a consistent pattern The output decreased approximately 5 per cent over the first 4 hours after awakening and continued at this baseline level through the day and that night usually showing a low grade rise between 5 and 7 A M The following studies of carbon dioxide production are presented in terms of the stimulation to increases in carbon dioxide output above fasting levels associated with 3 normal meals and with the same food introduced as tube feeding at varying rates

Four subjects were studied for 10 days when given normal meals and showed relatively marked increases in carbon dioxide output after each meal reaching 15 per cent to 20 per cent above baseline values with a variable tendency toward returning to baseline values between meals The baseline is reached sometime between 3 and 5 hours after the evening meal and remains at this level until after breakfast the following morning

In 2 patients who received the same food given as 8 equal meals over 12 hours the carbon dioxide output rose steadily over the period of the meals

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nitrogen retention. Even here a negative balance on the first and second postoperative days cannot be avoided. The data of Kosteritz[6] are impressive: he found that rats transferred from a stock diet to a protein free diet lost 16.2 per cent of their liver protein in 1 day and 23.8 per cent in 2 days. Subsequent daily losses were less. However, it is obvious that animals on depletion diets or patients unable to obtain protein rapidly lose nitrogen.

The present series of experiments shows that animals respond to decreased protein intake by reducing the rate of plasma protein turnover. No data are available on the size of the protein pools at the beginning and the end of the experiment, although they probably would have dropped slightly. In this case the rates of synthesis and catabolism would be even slower than indicated in Table 1, since smaller pools would in involved. Peters[7] has reported that the malnourished patient conserves nitrogen better than the normal individual.

The marked ability of depleted but otherwise normal dogs to double the

*Table 1. Turnover rates for various experiments and the average daily percentage of turnover in the various groups*

| GROUP | TYPE EXPERIMENT | EXPERIMENT NUMBER | HALF LIFE IN DAYS ALBUMIN | GLOBULIN |
|---|---|---|---|---|
| A | NORMAL ENDOGENOUS | A 1 | 13.0 | 8.3 |
| | | A 10 | 19.5 | 7.9 |
| | | A 6 | 13.0 | 8.7 |
| | | A 11 | 13.1 | 8.2 |
| | | A 12 | 12.6 | 8.0 |
| | | AVERAGE | 14.2 | 8.2 |
| | | DAILY PERCENTAGE | 4.9 | 8.4 |
| B | NORMAL EXOGENOUS | A 13 | 10.2 | 6.6 |
| | | A 17 | 9.1 | 4.5 |
| | | A 18 | 10.0 | 5.5 |
| | | AVERAGE | 9.8 | 5.5 |
| | | DAILY PERCENTAGE | 7.1 | 12.6 |
| C | DEPLETED ENDOGENOUS | A 16 | | 14.7 |
| | | DAILY PERCENTAGE | | 4.7 |
| D | DEPLETED EXOGENOUS | A 3 | 9.0 | 5.6 |
| | | A 14 | 11.5 | 6.9 |
| | | A 19 | 11.0 | 7.0 |
| | | AVERAGE | 10.5 | 6.5 |
| | | DAILY PERCENTAGE | 6.6 | 10.7 |
| E | REPLETED ENDOGENOUS | A 20 | 6.6 | 5.9 |
| | | A 21 | 7.1 | 6.2 |
| | | AVERAGE | 6.9 | 6.1 |
| | | DAILY PERCENTAGE | 10.0 | 11.4 |

250 ml of blood was withdrawn from dogs A-6, A-11 and A-12 on the sixth day of the experiments. This did not change the turnover curves.

protein intake does affect the rate of plasma protein turnover This effect is most pronounced in the previously protein starved animal

## METHOD

The details of plasma fractionation and the $S^{35}$ assay procedure have been previously described[1] The types of experiments are also similar and will be divided into 5 groups as shown in Table I The term endogenous refers to the protein synthesized by the animal after feeding the $S^{35}$ labeled yeast Exogenous refers to protein transfused in whole blood from a labeled donor dog The Group A dogs were maintained on a stock diet and on day zero received a dose of $S^{35}$ labeled yeast* mixed with dog meat Blood samples of approximately 20 ml each were drawn periodically on succeeding days for about 3 weeks and the albumin and total globulin fractions were assayed for $S^{35}$ specific activity as barium sulfate Dogs in Group B, also maintained on a stock diet received a transfusion of 250 ml of labeled whole blood from donor dogs on day zero During transfusion an equal volume of blood was drawn from a contralateral vein Group C and D dogs were treated identically with those in Groups A and B respectively except that they had been maintained on the low proten diet of Weech *et al*[3] for periods of from 6 to 10 weeks prior to the beginning of the experiment The Group E dogs had been on the low protein diet for 6 months On the day prior to the start of the experiment a stock diet plus supplemental meat feedings was started

## RESULTS

The results of the various experiments including those previously reported are summarized in Table I The rates for all dogs within a group agree well except for the albumin in dog A 10 Such occasional variations have been noted by others[4] The percentage of daily turnover is derived from the graphically determined half life by the following expression

$$\text{Percentage daily turnover} = \frac{\ln 2}{t\,1/2} \times 100$$

If the group comparisons are made A against B and C against D it is seen that exogenous proteins turn over more rapidly than endogenous This is true in both the albumin and globulin fractions for both normal and depleted animals although Group C contains only one determination

Comparing Groups A against C and B against D a definite tendency is seen for a slower rate of turnover in protein depleted animals With the repletion of a previously depleted dog a striking difference is noted The turnover rates in Group E are considerably faster than the normals and the globulin turnover rate is more than twice as fast as that of the comparable depleted animal Group C

## DISCUSSION

The importance of protein metabolism in surgery has recently been stressed by Cole *et al*[5] Describing inadequate intake they state that With out question this is the most common cause of hypoproteinemia Their data on post surgical patients indicate that ambulation and a diet of basal plus 20 per cent consisting of 40 per cent protein achieves the maximum

*Obtained from the Division of Radioactive Pharmaceuticals Abbott Laboratories

nitrogen retention. Even here a negative balance on the first and second postoperative days cannot be avoided. The data of Kosteritz[6] are impressive: he found that rats transferred from a stock diet to a protein free diet lost 16.2 per cent of their liver protein in 1 day and 23.8 per cent in 2 days. Subsequent daily losses were less. However, it is obvious that animals on depletion diets or patients unable to obtain protein rapidly lose nitrogen.

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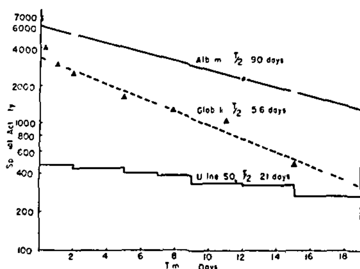



Fig. 1. Turnover curves for albumin, globulin and urinary $SO_4$ in a dog (A 3) *receiving a transfusion of* labeled whole blood. Specific activity is plotted logarithmically against time.

rate of endogenous albumin turnover during repletion is significant. In these *dogs the more rapid fall in specific activity results from increasing amounts* of newly synthesized unlabeled albumin added to the circulation. Jeffay[9] has determined the hourly replacement rate of albumin in the rat on various protein diets. With inadequate diets the replacement rate was 3 to 4 mg./hr.; with adequate diets it was 5 to 6 mg./hr.; and with excessive diets it was 10 to 12 mg./hr. Previous data using nitrogen balance techniques showed protein conservation during times of protein depletion. The present data indicate that the rates of protein synthesis and breakdown (turnover) are decreased during protein depletion and increased during protein repletion. *Thus the results of the balance studies are partially elucidated by the* dynamic studies using the turnover method.

These data have been considered with a view of determining whether plasma proteins are converted directly to tissue protein without intermediation of free amino acids. Yuile *et al.*[8] have presented evidence supporting the view that direct conversion of parenterally administered plasma proteins to tissue proteins occurs. The low levels and the prolonged half life of excreted radioactive sulfur in relation to the injected proteins (Fig. 1) are compatible with the view of direct conversion of plasma proteins to more *stable tissue protein. However, the results are also compatible with the re*incorporation into tissue protein of free labelled amino acids resulting from the breakdown of the injected plasma proteins. In the previous discussion[1] this reutilization of isotope was offered as an explanation for the apparently slower turnover rates of endogenous plasma proteins. Further experiments will be necessary to distinguish between these possibilities.

SUMMARY

1. Exogenous plasma proteins have an apparent turnover rate greater than endogenously produced plasma protein.

2. When a dog is starved in protein it turns over transfused and probably its own plasma proteins more slowly.

3. If dogs previously starved in protein are placed on a high protein diet there is a marked increase in the turnover rates of their endogenous plasma proteins.

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# ACUTE DEPLETION OF GAMMA GLOBULIN IN THE DOG*

GEORGE L JORDAN JR OSCAR CREECH JR WILLIAM H BARNETT
ARIEL BARSELA AND MICHAEL E DEBAKEY

Patients with agammaglobulinemia or extreme degrees of hypogammaglobulinemia fail to show an immune response to many stimuli In such patients homotransplantation of skin may be performed successfully but it has not been possible to ascertain with certainty that successful homotransplantation results from the absence of gammaglobulin rather than from some other defect in the immune mechanism - A review of the literature failed to reveal reports of the experimental production of agammaglobulinemia in animals However there are many agents which are known to affect the response to antigenic substances cortisone and nitrogen mustard are two such agents While neither has been reported to produce extreme degrees of hypogammaglobulinemia both appear to inhibit the production of antibodies[3 4]

This study was instituted in an attempt to produce a reversible agammaglobulinemia in the dog by acute depletion of gammaglobulin with exchange transfusions and inhibition of the production of gammaglobulin with cortisone and/or nitrogen mustard The accomplishment of this objective would provide a valuable experimental tool for studies of the effects of hypogammaglobulinemia

## METHOD

Mongrel dogs were chosen as the experimental animal Agammaglobulinemic blood was prepared by reconstituting washed canine red cells with agammaglobulinemic canine plasma (a by product of the commercial production of gammaglobulin for veterinary use) which contained an essentially normal distribution of other protein fractions and electrolytes † The serum levels of gammaglobulin were determined electrophoretically using the Tiselius apparatus and by the Kunkel test Comparison of results of these 2 methods in our laboratory revealed good correlation, but in our hands the

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†The Biological Laboratories of Pittman Moore Company Indianapolis Ind

test was less sensitive than reported by others, so that one Kunkel unit represented approximately 100 mg per cent serum gammaglobulin and 10 units approximately 600 mg per cent Normal Kunkel values ranged from 5 to 20 units in the animals studied

To compare the effect of depletion of total gammaglobulin with that of an induced antibody response animals were immunized with typhoid vaccine to titers of 1 640 and the changes in typhoid titer determined, using a test tube technique

Transfusions were performed by inserting polyethylene catheters into the femoral artery and vein under nembutal anesthesia Two types of transfusion procedures were used In the first type animals were bled from the femoral artery until bleeding ceased and the quantity of blood lost immediately replaced under pressure through the femoral vein catheter This procedure required approximately 10 minutes In the second type an estimated 25 per cent of the blood volume was removed through the femoral artery and this amount replaced by transfusion following which time aliquots of 15 or 20 ml of blood were alternately transfused and bled until a total of one to 1½ times the estimated blood volume had been exchanged This procedure required 30 to 90 minutes

Nineteen transfusions were completed in 11 untreated animals and 36 in 11 treated animals In each experiment the serum level of gammaglobulin was determined before and 15 minutes following the completion of the exchange by one or both of the methods described above Eleven of the transfusions in the untreated animals were of the first type described above including 3 in an immunized dog while 8 were slow exchanges In 5 of the latter experiments Kunkel tests were performed daily in the post transfusion period to follow the return to pre transfusion levels

Nineteen agammaglobulinemic transfusions were completed in animals treated with daily administration of 6 to 15 mg of cortisone per kg of body weight the drug being given at least 48 hours prior to the first transfusion and continued throughout the duration of the experiment The return to pre transfusion levels of serum gammaglobulin was recorded by daily Kunkel tests in 8 experiments Also 5 transfusions were performed in cortisone treated immunized animals and the immediate change in typhoid titer and the return to pre transfusion levels were recorded

Four transfusions were performed 3 days after a single dose of nitrogen mustard of 25 mg or 5 mg per kg of body weight and in 3 experiments the return to normal levels recorded Two additional experiments were performed in immunized animals

Six transfusions were completed in animals treated with both cortisone and nitrogen mustard and the return to normal levels recorded following 4 of these Two of these animals had received cortisone daily for 5 to 7 weeks prior to the administration of nitrogen mustard

## RESULTS

Three of the 4 untreated animals subjected to exsanguination and transfusion with agammaglobulinemic blood failed to show a significant decrease in the serum level of gammaglobulin at 15 minutes post transfusion and this type of transfusion also failed to reduce the typhoid titer in the immunized animal (Fig 1) The 1 animal which failed to maintain its pre transfusion

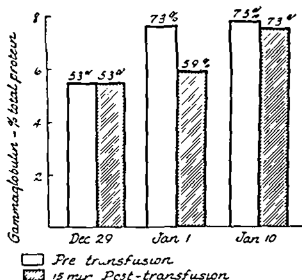



Fig 1 Failure of exsanguination and transfusion with agammaglobulinemic blood to significantly influence the serum level of gammaglobulin

level in the immediate post transfusion period died a few moments after completion of the experiment Exchange transfusions by the slower method resulted in some depletion of gammaglobulin in each of 8 experiments but the return to pre transfusion levels was very rapid (Table 1)

*Table 1 Acute Depletion of Gammaglobulin by Exchange Transfusion in an Untreated Animal*

| | TOTAL PROTEIN GRAMS % | GAMMAGLOBULIN % TOTAL PROTEIN | GAMMAGLOBULIN MGM % | GAMMAGLOBULIN KUNKEL UNITS |
|---|---|---|---|---|
| Pre transfusion | 5 0 | 6 7 | 335 | 5.3 |
| 15 min Post transfusion | 3.5 | 3 9 | 140 | 1.3 |

One transfusion of a treated animal usually produced a 50 per cent to 75 per cent reduction in the serum level of gammaglobulin and when transfusions were repeated daily a progressive lowering was recorded In 1 cortisone treated animal complete agammaglobulinemia was recorded electrophoretically after 3 successive transfusions

The average time required for the serum gammaglobulin level to return to that recorded prior to transfusion was longer in the animals treated with cortisone or nitrogen mustard than in the untreated animals and the combined effect of these 2 drugs was greater than that of either used alone However the range in the treated animals overlapped to some extent that of the untreated group and no animal remained hypogammaglobulinemic for more than a few days except 1 animal treated with cortisone and nitrogen mustard that died on the fifth post transfusion day of pneumonia On the day of death the serum gammaglobulin level was 1 4 Kunkel units (Table 2)

Typhoid titers were reduced by exchange transfusion but returned to pre transfusion levels at a slower rate average 13 days in the cortisone treated group and 9 days in the nitrogen mustard group Transfusions of whole blood from non immunized animals containing a normal quantity of gammaglobulin but no detectable typhoid antibodies resulted in the same de

*Table 2 Interval Required for Return of Gamma globulin to Pre transfusion Values Following Acute Depletion by Exchange Transfusion*

| GROUP | | DAYS RANGE | DAYS AVERAGE |
|---|---|---|---|
| A | Control | 1 5 | 2 |
| B | Treated | | |
| | 1 Cortisone | 1 15 | [illegible] |
| | 2 Nitrogen mustard | 2 7 | 4 |
| | 3 Cortisone and nitrogen mustard | 4 11 | 7 |
| | Total treated | 1 15 | 5 |

crease in typhoid titer as that observed after transfusions with agamma globulinemic blood and there was no difference in the rate of return to pre transfusion levels

The production of the hypoglobulinemic state appeared to serve as a stimulus to excess production of gammaglobulin as in many experiments the serum level rose higher than that recorded before transfusion (Fig 2)

DISCUSSION

The exsanguinating type of exchange resulted in an immediate replacement of approximately 80 per cent of the total blood volume with agamma globulinemic blood Therefore failure to significantly lower serum levels of gammaglobulin in this manner is confirmation of other studies leading to the conclusion that a large pool of extravascular protein is normally present which is in dynamic equilibrium with intravascular protein[1] The decrease which followed the slower type of exchange is probably due partly to the larger volume of the exchange but in addition it is likely that the slower exchange allowed time for mobilization of extravascular reserves so that this pool was depleted also Undoubtedly reduction to extreme levels of hypo gammaglobulinemia could be accomplished by increasing the total volume of one exchange but multiple transfusions of limited quantity were tolerated better

The fact that post transfusion values often exceeded pre transfusion values is considered evidence that the return to normal requires production of new

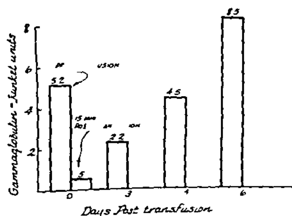



Fig 2 Rapid production of gamma globulin following acute depletion in animal treated with cortisone and nitrogen mustard

gammaglobulin and that acute depletion acts as a major stimulus Whereas *cortisone and nitrogen mustard retard the synthesis of gammaglobulin their* effect is limited so their use does not permit prolonged studies of the hypo gammaglobulinemic state However, the production of typhoid antibodies is much slower than the production of total gammaglobulin and the titer may be kept at low levels by repeated exchange transfusions in treated animals

SUMMARY

1 Acute depletion of gammaglobulin in the dog can be accomplished by exchange transfusion however this animal has a large reserve store which can be mobilized rapidly and production of new gammaglobulin occurs at *a rapid rate*

2 In comparison to the results in untreated animals cortisone and nitro gen mustard retard the return of serum gammaglobulin to pre transfusion levels

3 Typhoid antibodies are produced more slowly after acute depletion than is total gammaglobulin

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# EXPERIMENTAL GRAFTING OF A SUSPENSION OF SKIN PARTICLES*

JOHN S NAJARIAN AND H J MCCORKLE

The covering with epithelium of denuded areas resulting from the extensive loss of skin—particularly following severe thermal injuries—has long been a difficult surgical problem[1] In such cases fresh and stored homografts have been used as a temporary epithelial covering[2] More permanent results have been achieved with patch grafts[3] or split thickness grafts removed at repeated intervals from the donor areas[4]

The following experiments were performed in an effort to produce epithe lial covering over widely denuded areas with the removal of relatively small amounts of skin from the available areas of intact skin

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Supported by the Christine Breon Fund for Medical Research

*Table 2 Interval Required for Return of Gamma globulin to Pre transfusion Values Following Acute Depletion by Exchange Transfusion*

| GROUP | DAYS RANGE | DAYS AVERAGE |
|---|---|---|
| A Control | 1 5 | 2 |
| B Treated | | |
| 1 Cortisone | 1 15 | 5 |
| 2 Nitrogen mustard | 2 7 | 4 |
| 3 Cortisone and nitrogen mustard | 4 11 | 7 |
| Total treated | 1 15 | 5 |

crease in typhoid titer as that observed after transfusions with agamma globulinemic blood and there was no difference in the rate of return to pre transfusion levels

The production of the hypoglobulinemic state appeared to serve as a stimulus to excess production of gammaglobulin as in many experiments the serum level rose higher than that recorded before transfusion (Fig 2)

DISCUSSION

The exsanguinating type of exchange resulted in an immediate replace ment of approximately 80 per cent of the total blood volume with agamma globulinemic blood Therefore failure to significantly lower serum levels of gammaglobulin in this manner is confirmation of other studies leading to the conclusion that a large pool of extravascular protein is normally present which is in dynamic equilibrium with intravascular protein [1] The decrease which followed the slower type of exchange is probably due partly to the larger volume of the exchange but in addition it is likely that the slower exchange allowed time for mobilization of extravascular reserves so that this pool was depleted also Undoubtedly reduction to extreme levels of hypo gammaglobulinemia could be accomplished by increasing the total volume of one exchange but multiple transfusions of limited quantity were toler ated better

The fact that post transfusion values often exceeded pre transfusion values is considered evidence that the return to normal requires production of new

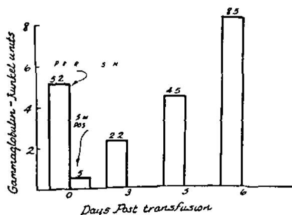



Fig 2 Rapid production of gamma globulin following acute depletion in animal treated with cortisone and nitrogen mustard

split thickness skin graft was obtained from the excised skin mounted on a block and divided into a suspension of skin particles as previously described The fine mesh gauze with the film of skin particles was then placed directly over the fascia of the paravertebral muscles The same type of dressing used for the animals in the first series was applied and left in place until the fourteenth day

In group 3 similar full thickness skin defects of the same size were made on 4 rabbits No skin grafts were applied but the dressings were the same as those used in groups 1 and 2 These animals were used as controls and also to observe the rate of contracture of such defects in the absence of skin grafts

In all series of experiments the suspensions of skin particles were stained with Papanicolaou s stain for histological study of the size and nature of the divided skin particles

## RESULTS

Successful grafts were obtained in all but 3 of the 32 rabbits grafted The 3 unsuccessful grafts resulted from technical errors in the preliminary experiments

Inspection of the grafted site 2 weeks postoperatively revealed numerous scattered islands of epithelium (Fig 1a) These islands of epithelium through proliferation usually coalesced to completely cover the entire site by the third postoperative week (Fig 1b) The survival of grafts and rate of complete epithelialization was essentially the same whether they were applied to granulation tissue or directly on fascia

Because the grafts were so thin contraction was apparent in all instances However contraction of the grafts applied over granulation tissue was more

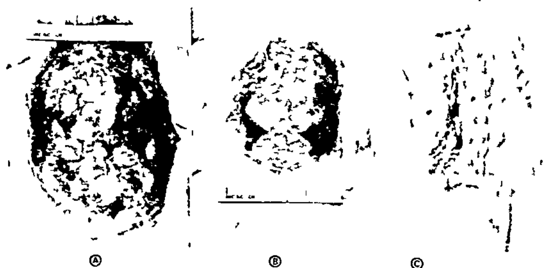



Fig 1 Photograph of rabbit s back showing (a) a recipient area 14 days after grafting the suspension of skin particles Numerous scattered islets of proliferating epithelium are seen throughout the operative field (b) Operative field 21 days after grafting the suspension of skin particles The islets of epithelium have coalesced to form almost complete covering of the recipient area (c) 5 weeks after a similar defect was made in another rabbit without applying a skin graft There is almost complete obliteration of the defect with a stellate scar

## METHOD

Thirty six adult rabbits weighing from 5 to 7 pounds were used in this study General anesthesia was produced with intravenous pentobarbital sodium solution and all procedures were performed with strict aseptic precautions The suspension of skin particles was prepared with an electrical kitchen blender that has two component parts a glass container and a base containing the electrical motor The instrument functions by the action of 2 metal blades spinning rapidly on the bottom of the glass container The glass container can be either autoclaved or cold sterilized with quarternary ammonium chlorides

**Preparation of the Grafts** A split thickness skin graft was placed in the sterile container of the blender and enough cold normal saline was added to bring the skin graft into contact with the whirling blades The minimum amount of normal saline added for this purpose was just sufficient to cover the blades lesser amounts were found to be ineffective in bringing the skin graft into contact with the blades It was necessary to cool the saline to 0 to 10 C in order to dissipate the heat produced by the blender The blender was operated at high speed for a period of 8 to 10 minutes with frequent observation of the temperature of the suspension throughout the procedure and addition of cold saline if the temperature rose above 37°C The suspension of skin was then transferred from the container to a sterile beaker on the operating table It was then applied with a sterile eye dropper or syringe to a piece of fine mesh gauze that had been previously cut to fit the area to be grafted The fine mesh gauze acts as a filter removing the saline solution and allowing the skin particles to be deposited as a thin layer over one surface of the gauze The gauze was then inverted and placed over the recipient area with the film of skin in contact with the area to be grafted

**Method of Transplantation** Two series of experiments were conducted In one the grafts were applied to freshly denuded fascia and in the others to granulation tissue Comparison was made between the two groups with regard to the survival of the graft and the contraction that occurred

In the first group an area of approximately 10 by 12 cm of skin surface was excised down to the fascia overlying the paravertebral muscles from the backs of 12 rabbits Fine mesh gauze impregnated with nitrofurazone ointment was placed directly over the defect A dressing was applied using several thicknesses of surgical gauze held firmly in position with a thick piece of cotton cloth sutured to the skin margins at the periphery of the excised area This dressing was changed after one week At the end of the second week the dorsum of the ear was prepared as a donor site and a split thickness of skin approximately 2 x 4 cm in size was removed with a skin grafting knife This graft was then prepared as previously described and the fine mesh gauze with the film of skin particles was placed over the area of granulation A similar dressing was used as before with the addition of a fine catheter incorporated into the surgical gauze This catheter was used during the ensuing two weeks for the introduction of 20 to 30 cc of normal saline solution daily to keep the dressing moist The dressings were removed on the fourteenth postoperative day

In the second group an area consisting of the full thickness of skin measuring not less than 8 by 10 cm was excised from the backs of 20 rabbits A

by this method would make its use undesirable in all but the most severe cases However if done successfully it would be an adequate temporary biological dressing for an extensively burned patient until definitive surgical repair could be done The simplicity of this method of preparation and application of skin in the preliminary treatment of large denuded areas might be advantageous for use in isolated areas as well as in large medical centers

SUMMARY

A method of preparation and grafting of a suspension of skin particles on rabbits is described Ninety per cent successful grafts were obtained when they were applied by this method to either granulating wounds or directly on fascia The possibility of the application of this method of skin grafting to human patients is suggested

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# DEVELOPMENT OF HISTOLOGICAL TECHNIQUES FOR THE DIFFERENTIAL STAINING OF BURNED AND NORMAL TISSUE*

J RAYMOND HINSHAW AND HERMAN E PEARSE

Much experimental work on burns has been hampered by the inability of an investigator to determine precisely the depth of injury produced Comparisons of the effects of various exposure times and irradiances comparisons of the ability of different agents to protect against thermal energy and predictions of heat flow through tissue and thermal damage to tissue are dependent on the demonstration of the level at which destruction ceases and normal structures remain The common histologic techniques for staining experimentally burned skin necessitate a highly subjective interpretation of the depth of injury The investigator must be experienced in histology and able to distinguish between minute differences in cell composition and shape and two such experts do not always agree on the result

A search was made for staining techniques which would yield a striking color contrast between normal and burned tissues It was borne in mind that these methods to be most useful should be relatively simple and repro

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marked than that observed when freshly denuded fascia was used as a recipient site This difference in size may possibly be due to the added time factor of 2 weeks during which the inflammatory process associated with the formation of granulation tissue was developing In the animals without grafts the defect contracted down to complete obliteration within 5 weeks leaving a stellate scar (Fig 1c)

Histological examination of the suspended skin particles revealed that the blender had divided the skin into clumps of epidermal cells collagenous fibers hair shafts and hair follicles The epidermal cell groups varied from 10 to about 100 cells The 4 major types of epidermal cells varying from the basal cells found in the lower malpighian layer to the anucleic cornified squamous cells are represented in Figure 2 Proliferation of epidermis can be expected from the basal cells and hair follicles and some growth potential appears to be present in the precornified epidermal cells

It seems possible that this method of grafting skin may be applied to patients with thermal injuries involving extensive loss of skin and in whom the remaining skin is not sufficient to make suitable grafts by other means The contracture of skin that may result from the extremely thin grafts applied

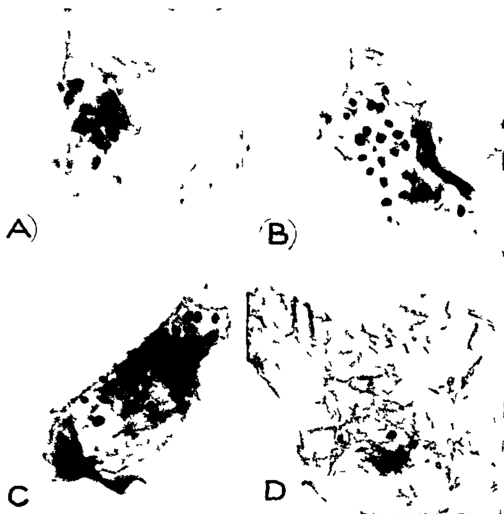



Fig 2 Photomicrographs of a suspension of rabbit skin particles with representative groups of (a) basal (b) precornified (c) follicular and (d) cornified epithelial cells



Fig 1 Burned porcine skin stained with hematoxylin and eosin Normal dermis on the right burned dermis on the left × 20

burned from normal tissues This technique a modification of Verhoeff s methods stains the tissues with a mixture of hematoxylin ferric chloride, iodine and potassium iodide They are differentiated in aqueous ferric chloride and counterstained lightly with Van Gieson s stain This produces a marked color difference between burned and normal tissues (Figs 2 and 3) and the results are so consistent that duplicate staining is not necessary Normal epidermis is gray burned epidermis is yellow Normal dermal collagen is yellow and burned collagen is black or purple

DISCUSSION

In many experiments with burns it is necessary to determine as accurately as possible the depth of injury resulting from a particular thermal insult In this laboratory specimens of more than 3000 burns have been stained with hematoxylin and eosin With this technique if staining conditions are well



Fig 2 A section from the same block of tissue shown in Fig 1 but stained by the method described in this paper Note the marked contrast between burned black dermis on the left and normal light dermis on the right × 20



Fig 3 A partial thickness burn produced by a 0.3 second exposure to radiant thermal energy Stained by the method described × 150

ducible in any tissue laboratory and that workers with little training in micropathology should be able to interpret the results without difficulty Such techniques have been found and their reliability and accuracy have been demonstrated in well over a thousand experimental burns One of the most useful methods is described and illustrated here others will be reported subsequently

METHOD

The experimental animals were young Chester White pigs anesthetized with intraperitoneal Dial in Urea urethane (Ciba) in doses of 70 mg/kg of body weight The hair was removed with electric clippers and the skin was gently washed A heat source consisting of a modified 24 inch Army carbon arc searchlight[2, 5] produced burns which varied from mild erythema to severe full thickness damage The port which controlled the size of the burn was 1.7 cm in diameter Exposure times from 0.3 to 30.0 sec and irradiances from 32 cal/cm²/sec to 0.16 cal/cm²/sec were used

Eighteen to 24 hours after the burns were placed biopsies were taken across the injured areas so that normal tissue was included on each side of the burn The biopsies were fixed in 10 per cent formalin or Bouin's solution and embedded in paraffin

Various staining solutions were selected for trial because results from their use in standard histologic techniques led us to believe they might aid in the differential staining of burned skin When a technique offered promising results staining and differentiating times were altered to achieve ideal staining conditions Sections were then examined by both experienced and inexperienced microscopists to determine what color combinations could be interpreted most easily and most accurately The final step consisted of altering staining differentiating washing and counterstaining times so that the desired end result could be reached with large groups of slides and without individual attention to each slide

**Staining Method** Fix tissues in 10 per cent formalin and embed in paraffin To prepare the staining solution with the aid of heat dissolve 1 g hematoxylin (C C) in 20 ml absolute alcohol Filter and add 8 ml of a 10 per cent aqueous solution of $FeCl_3$ and 8 ml of an iodine solution made by dissolving 2 g iodine and 4 g KI in 100 ml of distilled water

Remove paraffin from the sections in the usual manner Immerse the sections in the above staining solution for 20 to 30 min Differentiate in a 2 per cent aqueous solution of $FeCl_3$ A rack containing about 25 slides can be dipped up and down in this solution 20 times or so in 20 to 30 sec Wash immediately and thoroughly in fresh tap water Place in 95 per cent alcohol for 1 min Wash in running tap water thoroughly for 5 min Counterstain for 15 sec in Van Gieson's stain Dip slide once in tap water Dehydrate in 95 per cent alcohol followed by absolute alcohol Clear in xylene and mount

These precautions should be observed The staining solution should be used within 24 hr and a fresh solution should be substituted after each 75 slides Differentiation in 2 per cent aqueous $FeCl_3$ is accomplished more rapidly than in the standard Verhoeff stain Until an exact time is established a single slide may be examined in water to determine the degree of differentiation Depending on the result the slides may be either restained or differentiated further The last washing in tap water must be thorough A short counterstaining time with Van Gieson's stain yields slides which may be easily interpreted The more brilliant colors produced by a longer counterstaining time are less easy to read

RESULTS

The result of the use of 1 of the 5 methods which have been developed will illustrate what can be expected of a stain in the differentiation of

# *Corticoid Changes During and Following Operation*

## THE DISTRIBUTION OF CORTICOSTEROIDS IN THE PLASMA AND RED CELLS OF SURGICAL PATIENTS*

ARNOLD MITTLEMAN AND HAROLD G. BARKER

The partition of corticosteroids between red cells and plasma of human blood is controversial but most investigators determine only concentration in plasma since plasma is a less troublesome medium to extract. Simpson and Tait[1] have added radioactive compound F and aldosterone to whole blood and found roughly 25 per cent of these materials in the red cells. Hecter *et al.*[2] have found significant quantities of added steroid in the red cells of perfusing media. On the other hand Morris *et al.*[3] and Nelson and Samuels[4] have found no adrenocortical steroids in the red cells of peripheral and adrenal vein blood. We have measured this partition in normal human blood and have also measured the effects upon it of diurnal variation and of operation.

### METHOD

In the determination of the normal partition we utilized a total of 2 liters of blood, each portion of which was freshly drawn and immediately processed as described below. The measurement of diurnal variation was carried out on 4 preoperative surgical patients from each of whom a 100 cc. blood sample was drawn both at 8 A.M. and 5 P.M. The study of the effects of surgery was conducted in the same 4 patients (3 herniorrhaphies and 1 cholecystectomy). Again, 100 cc. blood samples were utilized and these were drawn at 8 hr., 24 hr. and 72 hr. after operation. All bloods were drawn using heparin as the anticoagulant and centrifuged promptly (within 10 min.) in a water cooled Sorvall centrifuge at 8,000 rpm and the plasma promptly separated. The cells were then resuspended and separated twice using physiological saline solution in quantities equal to that of the original plasma. The aqueous fractions were added to the plasma and the red cells were lysed with an equal volume of distilled water.

The plasma and lysed red cell samples were then extracted by the alcohol precipitation method of Bongiovanni.[5] To insure completeness of extraction 2 warm alcohol and 1 butanol wash of the precipitated blood proteins were carried out and the combined extracts evaporated to dryness by nitrogen vacuum distillation in a 45 C. water bath. The dried extracts were suspended in distilled water, hydrolyzed with β glucuronidase (Ketodase) and subjected to continuous dialysis and extraction by the method of Lombardo *et al.*[6] The resulting extracts were chromatogrammed for 72 hr. with appropriate standards in toluene propyleneglycol at 30°C. The strip areas corre

* From the Department of Surgery, Columbia University College of Physicians and Surgeons, and the Surgical Service, Presbyterian Hospital, New York. This study was supported in part by U. S. Public Health Grant RG 2926 (C-4).

controlled there is a color difference between acidophilic burned epidermis and basophilic normal epidermis However when many slides are stained simultaneously even this difference is not consistent Mallory s stain and Masson s trichrome stain are of equally limited value With all 3 techniques one must determine the extent of injury by the size and shape of nuclei and by almost insignificant differences in the composition and color of collagen This is very time consuming and it is disconcerting how often experienced microscopists disagree on the findings

Because the method presented here and other methods to be reported subsequently provide such a marked color difference between normal and burned tissues the slides can be read easily and rapidly The accuracy of the methods has been checked by staining sections from the same blocks of tissue by a number of techniques Long careful examination of the hematoxylin and eosin sections and a much more rapid examination of the specially stained sections lead to virtually the same estimate of the depth of damage

The method reported here has already proved invaluable in experimental work on the effects of superimposing two radiant energy thermal burns[3] on the use of flash creams in protecting bare skin against radiant thermal energy[1] and on the prediction of the depth of injury by surface appearance of burns[4]

SUMMARY

1 A search was made for histologic techniques which would provide a striking color contrast between normal and burned tissues

2 One of the most useful techniques is described and illustrated

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less this is done there is risk of steroid entering the cells *in vitro* Since transfer in the opposite direction is unlikely it is advisable to wash the cells and preserve the wash solution for addition to the plasma prior to processing In this manner one recovers the trapped plasma and increases the yield permitting more accurate analyses for a given volume of whole blood drawn

Experimental work involving the determination of steroid levels following the administration of exogenous steroids is probably best done on whole blood rather than plasma alone Recently we have given 300 mg of hydrocortisone hemisuccinate intravenously to a patient and thereafter determined the plasma red cell partition on freshly drawn blood A considerable amount of material having the same running rate as compound F was found in the cells Likewise it is probably wise to use whole blood analyses whenever *in vitro* experiments are being done or if prompt separation of samples drawn from patients is not possible

SUMMARY

The plasma red cell partition of compound F and tetrahydro compounds has been determined in freshly drawn human blood under varying conditions The steroid was found to exist largely in the plasma fraction (although small amounts were found in the red cells) in normal resting individuals and also during the fluctuations in whole blood steroids which resulted from both diurnal variation and the operative stress reaction When exogenous steroids are administered however large quantities enter the red cell and under these conditions it is advisable to use whole blood for analysis The same is true whenever the cells remain in contact with the plasma for considerable periods *in vitro* such as when prompt separation is impractical or in the conduct of perfusion experiments

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*Table 1. Diurnal Variation in Blood Partition of Steroids. Mean Values* in 4 Patients*

| | PLASMA | | RBC | |
|---|---|---|---|---|
| TIME | COMP. F | TETRAHYDRO | COMP. F | TETRAHYDRO |
| A.M. | 27 | 35 | 6 | 9 |
| P.M. | 18 | 17 | 3 | 4 |

*Micrograms percent

sponding to compound F and the tetrahydro compounds were eluted and measured by the BTZ reaction (micro adaptation)[7] and the Porter-Silber reaction as modified by Bongiovanni using a Coleman Universal Spectrophotometer.

RESULTS

The 24 of freshly processed whole blood from normal humans showed compound F concentrations of 27.5 μg per cent in the plasma and 8.6 in the red cell portion. The tetrahydro compound concentrations were 35 in the plasma and 11.2 in the cellular portion. In the study of diurnal variation the mean values for the 4 unoperated patients showed an evening drop in compound F and tetrahydro compound concentrations in both the plasma and red cell portions. (See Table 1.) The study of the effect of the combined stress of anesthesia and operation showed that the mean values in the 4 patients rose well above the normal at 8 hr. and thereafter began a gradual decline but at 72 hr. the figures were still above normal. The changes were reflected in the concentrations of both compound F and tetrahydro compounds in both plasma and red cell fractions. (See Table 2.)

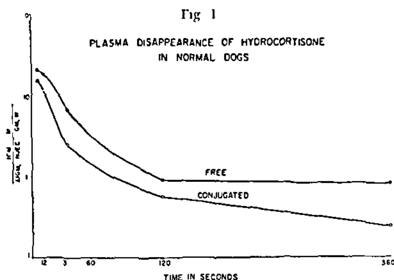
*Table 2. Postoperative Blood Partition of Steroids. Mean Values* in 4 Patients*

| | PLASMA | | RBC | |
|---|---|---|---|---|
| TIME | COMP. F | TETRAHYDRO | COMP. F | TETRAHYDRO |
| Preop | | See Table 1 | | |
| 8 hr. postop | 55 | 67 | 11 | 13 |
| 24 | 48 | 51 | 7 | 7 |
| 72 | 40 | 45 | 6 | 7 |

*Micrograms percent

DISCUSSION

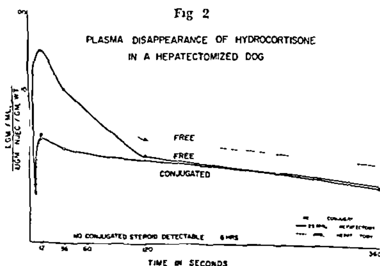
It seems evident from our studies that endogenously produced adrenocortical steroids circulate in the blood largely in the plasma fraction although small amounts do enter the red cells. Furthermore, fluctuations in total blood steroids with both diurnal variation and with the stimulation of operative stress are well reflected by the concentrations in plasma. Thus in conducting experimental work which deals only with endogenous steroids it is probably justified to continue the practice of limiting analyses to the plasma fraction. This is particularly true if the separation of plasma from cells is carried out promptly after the samples are drawn. There is evidence that un




plasma than from any other vein in the body indicating some extrahepatic conjugation Since this was not the anticipated finding the plasma was tested for completeness of extraction It was demonstrated that all of the chloroform extractable hydrocortisone was removed from the plasma prior to B glucuronidase extraction

In Figure 1 is shown a composite semilog plot from 5 normal dogs of the plasma disappearance and conjugation curves of instantaneously injected hydrocortisone The values obtained from the 5 animals were made comparable by expressing the concentrations as $\mu g/ml / \mu g$ injected per gram of body weight *The concentration of the conjugated steroid was extremely high in* the first pass arterial sample indicating very rapid conjugation of hydrocortisone The simultaneous mixing and disappearance of hydrocortisone and its conjugation curve is not well enough established at the present time to derive the number of exponential components

The plasma disappearance and conjugation curves of hydrocortisone in a dog 25 min following hepatectomy and 6 hr following hepatectomy are illustrated in Figure 2 The experiment demonstrates impaired steroid conjugation when no liver tissue is present However 6 hr following hepatec



# A PRELIMINARY STUDY OF THE DYNAMICS OF STEROID CONJUGATION*

M Don Turner James D Hardy and John O Damiler Jr

The chemical and physiochemical events that occur following the elaboration of the steroid hormones from the adrenal cortex are slowly being revealed However, the alterations which occur in these steroid hormones as they exert their influence upon the physiologic mechanisms of the organism and the actual nature of their action remain to be solved

It is known that the principal glucosteroid hydrocortisone, is secreted by the adrenal cortex into the circulation as the steroid alcohol A large part of the total hydrocortisone is readily converted to the tetrahydro form and excreted by the kidney as a glucuronide conjugate and a smaller portion as a sulfate conjugate *It appears that the conjugation process represents an* event or series of events which inactivates the steroid hormone and prepares it for rapid renal excretion The selective renal excretion of the conjugated form of the steroids is well established It is possible that the study of events leading up to conjugation will yield considerable information concerning the form of the steroid hormone at the instant of action

This paper is a preliminary report on a study of the dynamics of steroid conjugation

## METHOD

The method used for the determination of free and conjugated hydrocortisone blood levels were those of Nelson & Samuels[1] and of Bongiovanni

**Adrenal vein blood and analysis** Blood samples were obtained directly from the adrenal veins of 5 patients during suitable surgical procedures These adrenal vein blood samples were analyzed for the free alcohol of hydrocortisone and for the glucuronidate forms

**Plasma disappearance of free hydrocortisone and the appearance of conjugated hydrocortisone** Five milligrams of hydrocortisone was suddenly introduced into the venous circulation of 5 normal dogs Arterial samples were obtained at 6 12 36 60 120 360 sec and the plasma analyzed for both free and conjugated steroid

**Conjugation of hydrocortisone in the totally hepatectomized dog** A series of dogs was totally hepatectomized and the early plasma disappearance and conjugation curves of hydrocortisone were constructed for these animals at different time intervals following hepatectomy The curves were determined at 25 min 1 hr 3 hr and 6 hr following hepatectomy

## RESULTS

The human adrenal vein and systemic plasma levels of free hydrocortisone and of the glucuronidate form were measured in 5 patients The concentration of the conjugated hydrocortisone was much higher in adrenal vein

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Department of Surgery University of Mississippi Medical Center Jackson Mississippi
This work was *performed under Army Contract No DA 19 007 MD 627 Office of the Surgeon General* Department of the Army
With the technical assistance of Thelma Carter and Jennice Cooper

working hypothesis that the biological half life of hydrocortisone is approximately 3 hr. in the dog.


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# STEROID METABOLISM IN MAN: MINUTE HYDROCORTISONE OUTPUT OF LEFT ADRENAL*

*Preliminary Report*

JAMES D. HARDY AND M. DON TURNER

One of the requirements for successful surgery is that the patient have adequate adrenocortical function, and multiple studies have indicated that surgery usually increases corticoid production. Evidence has been derived by various means, the more direct being the measurement of blood corticoid levels and the daily urinary excretion of steroids in human beings. Hume and Nelson[1] actually measured hydrocortisone production in dogs by cannulating the adrenal vein following contralateral adrenalectomy. However, so far as we are aware, no studies of the steroid content of adrenal vein blood have previously been reported in the human. Thus, the purpose of this investigation has been to measure hydrocortisone concentration in adrenal vein blood samples taken at operation and, by means of timed adrenal vein blood flow, to effect an approximation for the output of hydrocortisone per minute. The concentrations of both the free and conjugated forms of the steroid have been measured in peripheral and adrenal venous blood samples taken simultaneously.

## METHOD

**Anatomic Considerations.** The venous drainage of the left adrenal gland courses largely through the central vein to enter the left renal vein approximately opposite the left ovarian vein in the female and the left spermatic vein in the male. This vessel is usually almost 2.5 cm. in length and from 2 to 4 mm. in diameter as it emerges from the adrenal gland. By inserting a No. 17 needle into the adrenal vein and temporarily occluding the vessel as it enters the renal vein, the blood flow through the former can be collected over a timed interval. To be sure, there exist patients in whom the adrenal is drained by several other smaller veins, in some of whom the flow by alter-

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*With the technical assistance of Thelma Carter and Virginia Ward.

tomy no conjugated steroid could be detected in the plasma after the intravenous introduction of the free form This has been demonstrated out to 6 hr in 2 dogs at the present time The conjugation mechanism loses most of its capacity immediately following hepatectomy and continues to decline to 6 hr when the conjugation process can no longer be detected

DISCUSSION

With the present method for the determination of the glucuronide conjugate of hydrocortisone [2] we obtained a peak of the conjugated form almost immediately following rapid injection of the alcohol of hydrocortisone If hydrocortisone is infused slowly over a period of approximately 15 min we obtain a peak of the conjugated form 1 hr from the beginning of the infusion This latter finding has been confirmed by a number of investigators The early conjugation peak was first thought to be an artefact Many attempts by the authors to disprove it have failed so far We are at the present time attempting to determine if the early peak conjugate is the *tetrahydro* form and if it is actually bound to the glucuronides

If the existence of this rapid conjugation peak is established it will throw some light on the dynamics of hydrocortisone in the circulation First of all the data indicate that the substance responsible for the conjugation mechanism is elaborated into the circulation by the normal liver For this reason conjugation proceeds not only in the liver but throughout the circulation Therefore conjugation of the steroid may be considered in part at least an extracellular process If it is true that any portion but not all of the hydrocortisone released by the adrenal cortex into the blood is instantly conjugated this indicates an enzymatic reaction which can approach completion only as the more soluble steroid conjugates are excreted by the kidney Since no conjugates were found 6 hr following hepatectomy even after the administration of 5 mg of hydrocortisone it is assumed that the turnover time of hydrocortisone is less than 6 hr in the dog This latter assumption represents a working hypothesis and certainly no definite conclusion can be drawn at the present time

The present data support the idea that the conjugation process acts as a safety mechanism to buffer the action of excess steroid and to enable the kidney to readily eliminate excess steroid by increasing the solubility of these materials in aqueous solutions

CONCLUSIONS

1 The conjugation of hydrocortisone with glucuronides occurs very rapidly A large portion of the hydrocortisone secreted by the adrenal gland is apparently conjugated at the instant of its release into the circulation Therefore at least a part of steroid conjugation occurs in the circulation and not in the liver

2 The presence of a normal liver is necessary for the conjugation process to continue However immediately following liver removal steroid conjugation still proceeds but at a rapidly declining rate until after 6 hr no conjugation occurs

3 Six hours following hepatectomy no glucuronide conjugate of hydrocortisone could be detected even after the administration of 5 mg of the hydrocortisone alcohol On the basis of this data the authors have set up a

working hypothesis that the biological half life of hydrocortisone is approximately 3 hr in the dog

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# STEROID METABOLISM IN MAN MINUTE HYDROCORTISONE OUTPUT OF LEFT ADRENAL*

*Preliminary Report*

JAMES D HARDY AND M DON TURNER

One of the requirements for successful surgery is that the patient have adequate adrenocortical function and multiple studies have indicated that surgery usually increases corticoid production Evidence has been derived by various means the more direct being the measurement of blood corticoid levels and the daily urinary excretion of steroids in human beings Hume and Nelson[1] actually measured hydrocortisone production in dogs by cannulating the adrenal vein following contralateral adrenalectomy However so far as we are aware no studies of the steroid content of adrenal vein blood have previously been reported in the human Thus the purpose of this investigation has been to measure hydrocortisone concentration in adrenal vein blood samples taken at operation and by means of timed adrenal vein blood flow to effect an approximation for the output of hydrocortisone per minute The concentrations of both the free and conjugated forms of the steroid have been measured in peripheral and adrenal venous blood samples taken simultaneously

## METHOD

**Anatomic Considerations** The venous drainage of the left adrenal gland courses largely through the central vein to enter the left renal vein approximately opposite the left ovarian vein in the female and the left spermatic vein in the male This vessel is usually almost 2 5 cm in length and from 2 to 4 mm in diameter as it emerges from the adrenal gland By inserting a No 17 needle into the adrenal vein and temporarily occluding the vessel as it enters the renal vein the blood flow through the former can be collected over a timed interval To be sure there exist patients in whom the adrenal is drained by several other smaller veins in some of whom the flow by alter

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nate channels may be considerable. Furthermore it may happen that the suction exerted on the needle introduced into the adrenal vein is such as to collapse the vein and thus to decrease the rate of flow per minute, in this case the blood that is obtained is relatively low in volume but high in corticoid content reflecting the fact that adrenocortical secretion is in this respect independent of the rate of blood flow.

The adrenal gland itself is approached through the gastrocolic ligament. The left kidney and the tail of the pancreas are first identified and the posterior parietal peritoneum overlying the pancreas is divided along the inferior border of the body and tail of the pancreas which is gently elevated and retracted cephalad with a Deaver retractor. This course of dissection permits exposure of the left renal vein, the superior mesenteric vein, and the splenic vein. Moreover by palpation in the space between the aorta and the superior pole of the left kidney the left adrenal gland can be identified. When the adrenal and the left renal vein have been adequately exposed the relatively large central adrenal vein is readily visualized and isolated.

The values for the concentrations of free and conjugated hydrocortisone in systemic, portal and adrenal venous blood were determined by the methods of Nelson and Samuels[2] and of Bongiovanni.[3] There was no significant difference between the concentrations in portal and those in systemic blood samples.

RESULTS

To date the analyses are complete in 10 patients and the values in a representative subject are shown in Figure 1. It may be seen that there was a prompt rise in both free and conjugated forms during operative stress followed by a decline after 4 hr. At the time when the systemic venous plasma levels of corticoids were 25.6 and 11.6 gamma/100 ml for free and conjugated forms respectively, the corresponding concentration for free hydrocortisone in adrenal vein blood was 312 gamma and for conjugated forms 90 gamma.

In the 10 patients the average measured adrenal vein blood flow was 25 cc./min., the average adrenal venous plasma level of free hydrocortisone was

SYSTEMIC AND ADRENAL PLASMA HYDROCORTISONE LEVELS

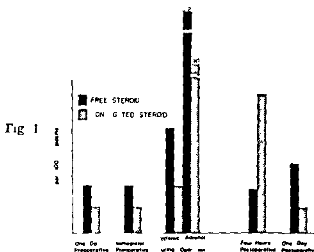



Fig. 1

224 gamma per cent and the average adrenal venous plasma level of conjugated hydrocortisone was 125 gamma. In systemic blood the average preoperative values for free and conjugated forms were 5.6 and 7.8 gamma/100 ml (8 patients), 21.0 and 16.7 gamma/100 ml respectively during operation (16 patients), and 9.9 and 25.2 gamma/100 ml 4 hr after the close of the operation (7 patients). The relative preponderance of conjugated forms postoperatively perhaps reflected the rapid conjugation of the large amount of free hydrocortisone secreted by the adrenals during operation. As a rule the rise in plasma corticoid levels occasioned by operation had subsided by the end of from 24 to 48 hr.

**Estimation of Hydrocortisone Output Per Minute.** Since the concentration of free hydrocortisone in systemic blood during operation averaged 21.0 gamma and that in adrenal venous blood 224 gamma/100 ml of plasma, the adrenal would appear to have effected an increase of 200 gamma per 100 cc of plasma flowing through it. Since the average plasma flow per minute was approximately 12.5 cc (total blood flow 25 cc), the output of free hydrocortisone per minute by the left adrenal may have been one eighth of 200 gamma or 25 gamma.

The minute output of conjugated hydrocortisone as reflected in adrenal venous blood was estimated in a similar manner. At the time when the plasma concentration of conjugated hydrocortisone in systemic venous blood was 16.7 gamma/100 ml, that in adrenal blood was 125 gamma, a difference of 108.5 gamma. If the adrenal plasma flow per minute was 12.5 cc, the secretion of conjugated forms per minute would appear to have been on the order of 13.6 gamma. Although it appears that the adrenal is elaborating a conjugated form, it is more probable that rapid conjugation occurs as the steroid enters the circulation.

In brief, on the basis of these data it is estimated that both adrenals contributed during operation a total of approximately 77 gamma of hydrocortisone per minute to the plasma, red cell content not considered. Furthermore, if the subject whose adrenal vein plasma flow was the greatest (56 cc/min) were to be considered separately, the hydrocortisone secretion per minute was on the order of 63.8 gamma free and 46.5 conjugated (Total = 110.3 gamma). It would appear that the selective increase of free as compared to conjugated hydrocortisone in systemic blood during operation is due to the rapid release of large quantities of the free alcohol by the stressed adrenals.

It is also estimated from the plasma data that the total hydrocortisone output per 24 hr is 34 mg. If one includes red cell hydrocortisone content estimated at 25 per cent of the total blood content by some workers, then the output per 24 hours might be 47 mg.

## SUMMARY


1. The effect of operation upon the systemic plasma levels of both free and conjugated hydrocortisone have been given.

2. By means of blood samples taken from the left adrenal vein at laparotomy an approximation for hydrocortisone secretion per minute has been made.

3. During operative stress in 10 patients the average total hydrocortisone secretion was estimated at 77 gamma per minute. In 1 patient who exhibited

the highest adrenal vein blood flow measured the value was on the order of 110 gamma per minute

4 The total hydrocortisone production in the non stressed subject is estimated at 47 mg /24 hr

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# THE EFFECT OF STIMULATION OF THE FEMORAL NERVE ON ADRENAL 17 HYDROXYCORTICOSTEROID SECRETION IN DOGS*

RICHARD H EGDAHL AND JOHN B RICHARDS

It has been suggested that nervous stimuli constitute an important if not crucial factor in the production of adrenocortical stimulation following trauma Long[1] has demonstrated adrenal ascorbic acid and cholesterol depletion following electrical stimulation of the central portions of exposed sciatic and brachial nerves in rats Hume and Wittenstein[2] have shown in dogs and Gordon[3] in rats that the application of certain stimuli (fracture operative trauma) to a limb results in adrenocortical stimulation and that *this response may be prevented by denervating the limb*

In the foregoing studies indirect indices of adrenocortical secretory activity were employed The present investigation was undertaken to study adrenocortical function following stimulation of the femoral nerve in dogs utilizing a technique which provides a specific and direct index of the secretory activity of the adrenal cortex and permits frequent sequential determinations in the same animal In addition the effect of induced high blood concentrations of 17 hydroxycorticosteroids upon the adrenocortical response to nerve excitation was studied

METHOD

Ten male mongrel dogs weighing 13 to 19 kg were used in this study The dogs were anesthetized with intravenous pentobarbital sodium and the right lumboadrenal vein of each animal was cannulated according to a technique described by Hume and Nelson [4] This preparation permits intermittent collection of venous blood from the cannulated adrenal gland The dogs were allowed to recover for a period of 36 to 48 hr after which they were re

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anesthetized with intravenous pentobarbital sodium and a femoral nerve in each dog was isolated and severed A period of 30 to 60 min was allowed to elapse before the collection of control adrenal vein blood samples was begun All adrenal blood samples were obtained by collecting the total venous effluent of the right adrenal gland for a period of one minute Following the collection of control blood samples the positive and negative leads from a 6 volt dry cell battery (Burgess 1FH) were placed on the surface of the isolated and exposed nerve for periods of 1 or 5 min The nerve became progressively charred during this procedure Adrenal venous blood samples were collected at frequent intervals following stimulation of the nerve In 5 of the 10 dogs the peripheral end of the severed nerve was stimulated and adrenal venous blood samples were obtained prior to stimulation of the central end of the nerve In the remaining 5 animals only the central portion of the severed nerve was stimulated In the first 3 dogs continuous mean arterial pressures were obtained throughout the experiment from a femoral arterial cannula connected to a strain gauge and recording potentiometer

In 3 dogs following the stimulation of the central end of the severed femoral nerve and collection of adrenal venous blood samples an intravenous infusion of 17 hydroxycorticosterone (Hydrocortone 100 mgm /1000 ml of 0 85 per cent sodium chloride solution 40 drops/min ) was administered for a period of 1 hr During the first 30 min of hormone infusion a number of adrenal venous blood samples were collected and at frequent intervals for the remaining 30 min Samples of femoral venous blood were obtained immediately prior to the hormone infusion period at the time of nerve stimulation and at the termination of the infusion All blood samples were analyzed for 17 hydroxycorticosteroid content by the method of Nelson and Samuels [5]

## RESULTS

Table 1 lists the adrenal 17 hydroxycorticosteroid output values obtained following femoral nerve stimulation in 7 dogs It can be seen that in the animals tested (Dogs 1 2 3 4 7) no significant change in adrenal 17 hydroxycorticosteroid secretion occurred following stimulation of the peripheral portion of the transected femoral nerve Stimulation of the central end of the nerve however resulted in a marked increase in adrenocortical secretory activity in all dogs This response was almost immediate with maximal 17 hydroxycorticosteroid output occurring within 5 min after the period of nerve excitation in 7 of 9 experiments The adrenocortical stimulation was of a transient nature and in most cases adrenal corticoid secretion reverted to control levels within 30 min No significant change in adrenal venous blood flow was noted In 3 animals continuous mean arterial pressures were obtained during the experiments The mean arterial pressures in animals 3 4 and 7 prior to nerve stimulation were 100 105 and 115 mm Hg respectively Femoral nerve stimulation was not accompanied by more than 5 mm Hg variations in mean arterial pressure in these dogs

Table 2 lists the results obtained from 3 dogs in which the effect of increased peripheral blood concentration of 17 hydroxycorticosteroids upon the adrenocortical response to nerve stimulation was studied In all 3 animals a marked increase in adrenal corticoid secretion was elicited by stimulation of the central portion of the femoral nerve despite the high plasma

*Table 1 The Effect of Stimulation of the Femoral Nerve on Adrenal 17 Hydroxycorticosteroid Secretion in Dogs*

| DOG NO | PERIOD OF NERVE STIMULATION (MIN) | CONTROLS No | CONTROLS Ave μg/min | ADRENAL VENOUS BLOOD 17 HYDROXYCORTICOSTEROID SECRETION μg/min MINUTES FOLLOWING STIMULATION OF NERVE — PERIPHERAL END 0 5 | 5 10 | 10 20 | 20 30 | CENTRAL END 0 5 | 5 10 | 10 15 | 15 20 | 20 30 | 30 60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | , | 0 5±0 8* | 0 2 | 0 3 | 2 8 | 1 8 | 11 1 | 11 6 | | 11 2 | 9 9 | |
| 2 | 1 | | 0 1±0 1 | 0 0† | 0 1 | 1 0 | 0 6 | 11 2 | 1 2 | 1 8 | 3 1 | 0 9 | |
| 3 | 1 | ( | 0 1±0 2 | 0 1 | 0 6 | 0 1 | 0 0 | 3 7 | 16 0 | 1, 1 | 10 2 | 10 2 | 0 8 |
| 4 | 1 | 3 | 1 9±0 7 | 1 1 | 0 5 | 2 2 | 0 6 | 12 2 | 5 2 | 1 6 | 0 0 | 1 6 | 1 1 |
| | 5 | 1 | 1 2±0 7 | | | | | 10 6 | 6 2 | 0 0 | 0 1 | 2 6 | 0 5 |
| , | 1 | 2 | 3 6±1 8 | | | | | 3 0 | 27 0 | 16 6 | 6 6 | ,6 | 5 2 |
| | 5 | 2 | 5 1±0 2 | | | | | 33 8 | 27 6 | 20 2 | 28 6 | 5 1 | 5 2 |
| 6 | 5 | 3 | 0 3±0 1 | | | | | 31 1 | 32 8 | 2, 8 | 2, 8 | 9 6 | 5 0 |
| 7 | 5 | 5 | 1 5±1 7 | 0 8 | 2 2 | 0 1 | 0 0 | 12 3 | 9 1 | 2 0 | 1 7 | 7 1 | 5 1 |

*Standard Deviation

†Zero Numbers Represent Steroid Output Values Below Sensitivity of the Analytical Method (0 1) — 0 3 μg)

*Table 2 Effect of Stimulation of the Femoral Nerve on Adrenal 17 Hydroxycorticosteroid Secretion in Dogs with Increased Peripheral Blood Concentrations of 17 Hydroxycorticosterone Period of Nerve Stimulation—one minute*

| | ADRENAL VENOUS BLOOD 17 HYDROXYCORTICOSTEROID SECRETION µg/min | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | PERIOD OF 17 HYDROXYCORTICOSTERONE INFUSION | | | | | | | | |
| | CONTROLS | | MINUTES AFTER NERVE STIMULATION | | | | | | | CONTROLS | | MINUTES AFTER NERVE STIMULATION | | | | | | |
| DOG NO | NO | AV µg/min | 2 | 5 | 8 | 10 | 15 | 20 | 30 | NO | AV µg/min | 2 | 5 | 8 | 10 | 15 | 20 | 30 |
| 8 | 1 | 1 5±1 6* | 9 8 | 9 8 | 10 4 | 10 8 | 7 2 | 2 6 | 0 1 (3 0)† | 3 | 2 6±0 3 (34 0) | 14 8 | 14 2 | 10 8 | 3 8 | 3 2 | 1 2 | 2 6 (57 0) |
| 9 | 1 | 0 9±0 5 | 0 6 | 4 6 | 14 6 | 9 4 | 3 4 | 0 7 | 2 2 (5 0) | 1 | 2 2±0 6 (82 0) | 5 6 | 8 8 | 7 4 | 5 8 | 4 0 | 3 9 | 4 9 (114 0) |
| 10 | 3 | 3 3±2 7 | 0 8 | 1 2 | 4 0 | 4 0 | 1 8 | 1 0 | 0 2 (1 6) | 3 | 3 1±1 4 (60 0) | 5 8 | 7 5 | 8 8 | 13 0 | 18 6 | 20 0 | 5 8 |

* Standard Deviation

† Numbers enclosed by parentheses represent plasma concentrations of 17 hydroxycorticosteroids (µg/100 ml plasma) in peripheral blood

concentrations of 17 hydroxycorticosteroids in the circulating blood stream Furthermore in 2 animals (8 and 9) the same pattern and magnitude of adrenocortical response to nerve stimulation were observed in the periods prior to and during hormone infusion In animal 10 no appreciable increase in adrenal steroid output occurred following the initial nerve stimulation but a marked increase resulted from the second stimulation (during the hormone infusion period) This result might possibly have been due to improper electrode placement at the time of the initial stimulation

DISCUSSION

The principal steroid hormone secreted by the adrenal cortex of dogs is 17 hydroxycorticosterone [6, 7] The quantitative estimation of 17 hydroxycorticosteroids in adrenal venous blood of this animal (as performed in these studies) provides a direct and specific index of adrenal cortical secretory activity

Previous studies of adrenal cortical function following nerve stimulation include the additional variables and likely pituitary adrenal stimuli of increased blood pressure alterations of neural reflexes and changes in respiration These physiologic changes are accompaniments of electrical stimulation of a nerve Fortunately we are not limited to electrical nerve stimulation for thermal chemical and physical methods have been successfully used [8] Especially interesting is the work of Granit and Lundberg[9] who found that thermal stimulation of the central end of peripheral nerves in cats resulted in selective small fiber stimulation The stimulus used in the present study was most likely of a thermal nature for the nerve became progressively charred and warmed during the application of direct current An early direct electrical effect cannot be ruled out In the present study, two facts stand out clearly 1) The stimulus was definitely effective 2) It did not result in changes of blood pressure or respiratory movement Because of the absence of these other physiological phenomena it is felt that the method of nerve stimulation utilized was particularly satisfactory Although analysis of potentials coming from the nerve would be of great interest following this stimulation and delineation of thermal chemical, and electrical components of the stimulation mechanism would be desirable such data are not necessary to the conclusion that the stimulus as used resulted in a greatly increased adrenal cortical secretion without the blood pressure changes attendant upon electrical stimulation It is probable that such specific stimulatory effect was achieved because fewer and perhaps smaller nerve fibers were stimulated

The adrenal cortical response following stimulation of the central end of the femoral nerve is marked but transient It has been found that the response to thermal stimulation of nerves rapidly deteriorates [9] After the adrenal response has subsided there is an immediate return to high levels with further central nerve stimulation In at least 1 animal the 5 min stimulation resulted in a greater adrenal secretion than after 1 min

Stimulation of the peripheral end of the femoral nerve did not result in adrenal cortical stimulation in any instance Gordon[3] has postulated that the mechanism of adrenal cortical stimulation following scalding is a release of a humoral factor at the sight of the scald which in turn activates the pituitary adrenal system The lack of adrenal response following stimulation of

the peripheral segment of a severed nerve indicated that a local humoral agent capable of eliciting pituitary adrenal stimulation is not produced as a result of peripheral nerve stimulation in general This does not rule out, of course the possibility of central nerve stimulation leading to the release of a central nervous system neurohumor which causes release of pituitary ACTH Indeed this is most likely [10]

It has been suggested by Sayers and Sayers[11] that ACTH release is effected by the level of circulating adrenal cortical hormones and that as the blood level of such hormones decreases as a result of peripheral utilization more ACTH is released In the present study central nerve stimulation was performed in 3 dogs with induced high peripheral blood levels of 17 hydroxy corticosteroids A marked adrenal cortical stimulatory affect was observed following central nerve stimulation It is apparent that an increased blood level of adrenal steroid hormones does not inhibit the adrenal cortical response to peripheral nerve stimulation

## SUMMARY

Stimulation of the central end of a transected femoral nerve in anesthetized dogs results in a transient but marked increase in adrenal 17 hydroxycorticosteroid secretion A similar stimulation of the peripheral portion of the severed femoral nerve produced no detectable change in adrenal corticoid secretion The pattern and magnitude of adrenocortical response to neural stimulation are not altered by the presence of increased concentrations of 17 hydroxycorticosteroids in the circulating blood stream These experiments indicate that neural excitation may play a role in the production of the pituitary adrenal stimulation observed following trauma

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# A COMPARISON OF THE STEROID FORMATION BY INCUBATED ADRENAL SLICES OF HYPERTENSIVE PATIENTS AND NORMOTENSIVE PATIENTS*

D. Y. Cooper, J. Roberts, J. C. Touchstone and O. Rosenthal

Two years ago we presented to the College of Surgeons a method for the quantitative and qualitative assay of *in vitro* steroid formation by adrenal tissue from human patients. Since this report, adrenals from 16 patients adrenalectomized for the treatment of hypertension and from 6 patients operated upon for palliation of carcinoma of the breast or prostate have been analyzed. The number of cases appears to be sufficiently large for a preliminary statistical comparison of the 2 groups. In addition, adrenal tissue from 4 normotensive cancer free patients biopsied because of suspected adrenal disease was studied.

## METHOD

The technique described in the previous paper has been employed throughout this study[1] and therefore will be described only briefly here.

Adrenal glands were obtained at the first stage operation from patients with hypertension and cancer of the breast and prostate. Biopsy samples were taken in patients who had had no recent operation. This tissue was iced immediately and taken to a cold room at 4 C where the incubates were prepared. After removing the periadrenal fat the adrenal gland was weighed. A portion of the gland was saved for pathologic study. The remainder was cut into thin slices by means of the Stadie Riggs slicer. Approximately 1.0 gm. of these slices was placed into a flask containing 10 cc. of the patient's plasma drawn prior to operation. Streptomycin and penicillin were added to prevent bacterial growth. The flasks were then immersed into a constant temperature water bath at 37.5°C. and incubated for 24 hr. while a slow current of 95 per cent oxygen 5 per cent carbon dioxide passed continuously through the vessels. After completion of the incubation the tissue and plasma were homogenized with a glass homogenizer and the proteins precipitated with acetone.

After removal of the precipitated proteins by filtration, the acetone was removed by distillation *in vacuo*. The aqueous residue was extracted with ethyl acetate and chloroform. After washing the extract with dilute sodium hydroxide and water the organic phase was evaporated to dryness by distillation *in vacuo*.

The residue was chromatographed on filter paper by the technique of Burton, Zaffaroni and Keutmann.[2] A scheme of the procedure is shown in Figure 1.

The strips were read at 245 mμ in the Beckman spectrophotometer using an adaptor described by Tennent, Whitla and Florey.[3] Representative strips

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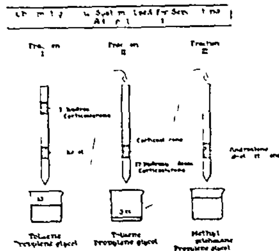


Fig 1 Scheme of the chromatographic separation of adrenal steroids Fraction I contains the Before F F and E ($\alpha$ ketol) regions Fraction II contains the B and S region Fraction III contains the $\Delta^4$ androstene 11$\beta$OH 3 17 androstene 11$\beta$ 3 17 dione and some of the B and S steroid that might run over from Fraction II

were sprayed with blue tetrazolium dye and reread at 600 m$\mu$ in the Beckman spectrophotometer

Absorption curves for all strips were obtained by plotting the optical density of each point against its distance from the starting line of the chromatogram Five definite areas or peaks resulted The first area noted after the starting line runs a little slower than Compound F and has been labelled before F area since the steroids present in this position have not been positively identified The second peak contains hydrocortisone (cortisol) which has been positively identified The third region studied is that in which Compound E is known to separate in this system Several steroid substances are present in this region but none of these materials has been identified This region has been labelled the E position The fourth peak is in fraction II and is the area in which known Compounds B and S would separate in this system Analysis of this position indicates that not only Compounds B and S are present but also additional unidentified steroid substances The fifth area contains $\Delta^4$ androstene 11$\beta$ OH 3 17 androstene 11$\beta$ 3 17 dione which has been positively identified in this system from extracts of human incubates[4]

Estimates of the quantity of steroid represented by each peak have been made by measuring the area under the curve of each ultraviolet peak and comparing this area with that produced by a standard amount of 11-desoxy corticosterone The values are expressed as $\mu$g of steroid per gram of adrenal tissue The results are summarized in Table 1

**Comparison of Steroid Formation by Adrenal Slices of Carcinoma and Hypertensive Patients** The chromatograms of the adrenal incubates from both groups of patients were identical with reference to number and position of absorption maxima seen Differences in the individual compounds that form the 5 areas studied may exist since of all the steroid compounds present only 2 have been positively identified

It is evident from Table 1 that there were striking differences between the groups in the quantity of steroids formed in these 5 positions The largest difference occurred in the B and S and 11 $\beta$ hydroxy androstenedione position The steroid formation in tissue from hypertensive patients averaged 198 and 209 $\mu$g/gm in the B and S and the 11 $\beta$ hydroxy positions respectively while corresponding means for the carcinoma patients were only 78 and 82 $\mu$g/gm

*Table 1  Steroid Formation (μg/gm) by Incubated Human Adrenal Slices*

Mean Values ± Standard Error
Number of Experiments in Parenthesis

| | WEIGHT | NO ACTH | | | | | 50 ACTH | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BEFORE F | F | E | B+S | 11 β hyd | BEFORE F | F | E | B+S | 11 β hyd |
| Normal | – | 81 | 226 | 118 | 90 | 82 | 116 | 385 | 181 | – | – |
| | (4) | (4) | (4) | (4) | (2) | (2) | (2) | (2) | (2) | – | – |
| Carcinoma | 490 | 59 | 192 | 61 | 78 | 82 | 140 | 358 | 91 | 162 | 171 |
| | ±21 | ±5 | ±29 | ±16 | ±19 | ±15 | ±30 | ±32 | ±12 | ±37 | ±33 |
| | (6) | (6) | (6) | (5) | (5) | (5) | (5) | (5) | (5) | (4) | (4) |
| Hypertension | 522 | 101† | 252† | 140† | 198† | 209† | 171 | 509† | 210† | 213* | 318 |
| | ±40 | ±12 | ±26 | ±10 | ±20 | ±22 | ±19 | ±59 | ±33 | ±30 | ±95 |
| | (16) | (16) | (16) | (13) | (9) | (7) | (14) | (14) | (11) | (7) | (4) |

*Significantly different from carcinoma group ($p < .05$)

†Significantly different from carcinoma group ($p < .01$)

A less striking but significant finding was the approximately 50 per cent increase in formation of the steroids of the before F and E positions by adrenal incubates from hypertensives. The mean steroid formation in these two positions averaged 42 μg/gm and 76 μg/gm greater than that in the incubates from cancer patients.

*The small difference between the mean values of cortisol formation by the* incubates from carcinoma and hypertensive patients resulted from the great variability of cortisol formation by incubates of hypertensive adrenals. Values for cortisol formation by these hypertensive adrenal slices varied from 150 μg/gm to 400 μg/gm.

Further study of the distribution of hydrocortisone data reveals that the *cortisol formation of hypertensive adrenals falls predominantly into two* definite groups: a low group forming 100 to 200 μg/gm of Compound F and a high group with values ranging from 300 to 400 μg/gm. This finding indicates that the hypertensive adrenals are not drawn from a homogeneous population, as will be discussed later on.

**Comparison of Hypertensive and Cancer Cases with Normotensive Cancer free Cases.** *In view of the small number of patients available in this group* no statistical analysis has been carried out on these data. Steroid formation by the incubates of this group resembled that of the cancer patients with the single exception of the steroid formation in the E position which was apparently greater.

**Effect of ACTH on the Incubates of Hypertensive and Cancer Patients.** *The addition of 5 units of ACTH to the incubates resulted in 2* changes in the general pattern of steroid formation by these adrenal incubates. No increase in formation above that found without ACTH was noted in the B and S position of hypertensive incubates. The average values of 16 hypertensive incubates for B and S steroid formation were 198 μg/gm before ACTH and 213 μg/gm after ACTH. A significant increase of from 78 μg/gm *to 198* μg/gm *was found in the carcinoma incubates*. In contrast stimulation of the E position in the incubates from carcinoma patients was small. Addition of 5 units of ACTH only increased the E position formation from 61 μg/gm to 91 μg/gm which was not significant statistically.

Increases of approximately 50 per cent were noted in the other positions after the addition of ACTH, as shown in Table I.

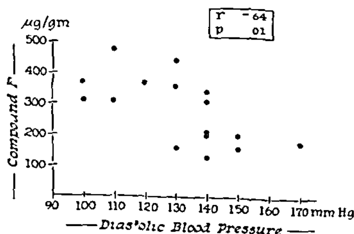



Fig. 2. The relationship between compound F formation in μg/gm and diastolic blood pressure.

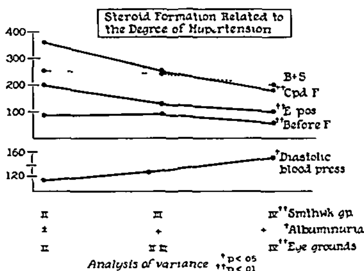



Fig 3 Relationship of formation of Compound F and of the steroids in the Before F E and B and S regions to increasing severity of hypertension *in 12 patients* Statistical significance was computed by the analysis of variance

**Relationship Between Steroid Formation in Adrenal Incubates and Clinical Hypertension** In an attempt to explain the separation of the cortisol formation into 2 definite groups the correlation of diastolic blood pressure to cortisone formation was studied A significant negative correlation ($r = -0\ 64$) was found as shown in Figure 2 This relationship indicates that a high diastolic blood pressure is associated with the formation of smaller quantities of cortisol per gram of incubated slices

In Figure 3 the formation of steroid in all positions has been related to diastolic blood pressure Smithwick classification albuminuria and eye ground changes It can be seen that a significant fall in Compound F (μg/gm) is associated with an increase in the severity of the hypertension as graded by the Smithwick classification Decreases in the steroid formation of the before F and E positions similar to that of cortisol were also associated with an increased severity of hypertension Significant increases of albuminuria and eye ground changes were also associated with decreased steroid formation as seen in Figure 3


SUMMARY

1 Mean values of steroid formation (μg/gm) by adrenal slices of 16 hypertensive patients were larger in all 5 areas of the chromatograms studied than that of adrenals from 6 patients with carcinoma Increased steroid formation was particularly pronounced on the B and S position

2 Steroid formation at the B and S position in adrenal incubates of hypertensive patients and at the E position of adrenal incubates of carcinoma patients did not increase with the addition of ACTH as the steroid formation of the other positions did

3 Cortisol formation by adrenal incubates of hypertensive patients fell into a low group (150–200 μg/gm) and a high group (300–400 μg/gm)

4 Decreased cortisol formation (μg/gm of adrenal tissue) was found associated with an increase in diastolic blood pressure increased severity of hypertension (according to the Smithwick classification) increasing albuminuria and increased severity of eye ground changes

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# ADRENAL CORTICOID OUTPUT IN SURGICAL PATIENTS WITH OBSTRUCTIVE JAUNDICE*

JOHN H SCHNEEWIND AND JOHN B FULLER

We have been studying urinary adrenal cortical hormone output in surgical patients in an attempt to assay the role of these vital substances in recovery following operation Surgical procedures activate the adrenal to an increased hormone output approximately 80 per cent of which in man is reported to be in the form of hydrocortisone—one of the 17 hydroxycorticoids The main functions of the 17 hydroxycorticoids are related to carbohydrate and protein metabolism and anti inflammatory and anti pyretic effects Regulation of electrolyte and fluid balance appears to be primarily affected by hormones without a hydroxy group in the 17 position such as aldosterone which are the minor fraction of adrenal output

Recent reports have called attention to the importance of the liver in the metabolism of the 17 hydroxycorticoids Peterson and associates[1] administered both hydrocortisone and radioactive hydrocortisone to normal and cirrhotic patients Following infusions of 200 mg of hydrocortisone free alcohol over a 20 to 30 min period to 18 normal patients they found that 50 per cent of the hydrocortisone had left the plasma in approximately 114 minutes When hydrocortisone was infused into 8 patients with cirrhosis it required from 160 to 800 min for half of the steroid to leave the plasma Other closely related steroids (cortisone tetrahydrocortisone etc ) did not show this delayed removal from the plasma These data were felt to imply that the enzyme system in the liver responsible for the metabolism of hydrocortisone was selectively impaired in liver disease Brown and associates[2] gave intravenous hydrocortisone to 11 normal patients and to 12 with liver disease Six hours after the infusion the plasma levels of the patients with liver disease was over three times as high as the normals The rate of disappearance of hydrocortisone was inversely proportional to the degree of liver damage as measured by BSP retention These authors also found that the patients with liver disease excreted about 30 per cent less 17 hydroxycorticoids in the urine

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*From the Department of Surgery University of Illinois Research and Educational Hospital Chicago Illinois These studies were supported in part by a grant from the Upjohn Company Kalamazoo Michigan We hereby express appreciation to Dr H C Batson for valuable assistance in analyzing our data statistically

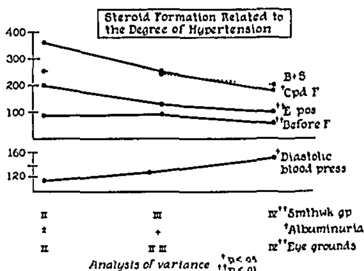



Fig. 3. Relationship of formation of Compound F and of the steroids in the Before F and B and S regions to increasing severity of hypertension in 12 patients. Statistical significance was computed by the analysis of variance.

**Relationship Between Steroid Formation in Adrenal Incubates and Clinical Hypertension.** In an attempt to explain the separation of the cortisol formation into 2 definite groups, the correlation of diastolic blood pressure to cortisone formation was studied. A significant negative correlation ($r = -0.61$) was found, as shown in Figure 2. This relationship indicates that a high diastolic blood pressure is associated with the formation of smaller quantities of cortisol per gram of incubated slices.

In Figure 3, the formation of steroid in all positions has been related to diastolic blood pressure, Smithwick classification, albuminuria and eye ground changes. It can be seen that a significant fall in Compound F ($\mu g/gm$) is associated with an increase in the severity of the hypertension as graded by the Smithwick classification. Decreases in the steroid formation of the before F and E positions similar to that of cortisol were also associated with an increased severity of hypertension. Significant increases of albuminuria and eye ground changes were also associated with decreased steroid formation as seen in Figure 3.

SUMMARY

1. Mean values of steroid formation ($\mu g/gm$) by adrenal slices of 16 hypertensive patients were larger in all 5 areas of the chromatograms studied than that of adrenals from 6 patients with carcinoma. Increased steroid formation was particularly pronounced on the B and S position.

2. Steroid formation at the B and S position in adrenal incubates of hypertensive patients and at the E position of adrenal incubates of carcinoma patients did not increase with the addition of ACTH as the steroid formation of the other positions did.

3. Cortisol formation by adrenal incubates of hypertensive patients fell into a low group (150–200 $\mu g/gm$) and a high group (300–400 $\mu g/gm$).

4. Decreased cortisol formation ($\mu g/gm$ of adrenal tissue) was found associated with an increase in diastolic blood pressure, increased severity of hypertension (according to the Smithwick classification), increasing albuminuria and increased severity of eye ground changes.

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in 24 hr than normals despite normal 8 A M plasma levels They concluded these data were evidence for decreased adrenocortical secretion in patients with liver disease in whom the rate of 17 hydroxycorticoid removal from plasma is impaired

We wondered whether similar evidence of disturbed steroid metabolism would appear in surgical patients with obstructive jaundice The data obtained from a study of 13 such patients as well as that on 6 normal patients (without evidence of obstructive jaundice or liver disease) form the basis of this report Also included are results on 4 cirrhotic patients subjected to operation

METHOD

Twenty four hour urines were collected from each patient for several days preoperatively during the day of operation and for the first 4 postoperative days

The method used for the determination of urinary total 17 OH corticoids is that of Reddy, Jenkins and Thorn [3] Steroids are extracted with butanol after adjustment to pH 1[4] and quantitated by the Porter Silber reaction

RESULTS

The data obtained indicate that the obstructive jaundice patients in this study excreted significantly smaller quantities of urinary 17 OH corticoids during the immediate postoperative period than did patients without obstructive jaundice or liver disease A fairly typical example of our findings in a jaundiced patient is shown in Figure 1 At operation a cholecystectomy and common duct exploration were performed Following a period of distention and ileus the patient suffered a wound dehiscence After repair he gradually improved and was discharged on the sixteenth postoperative day

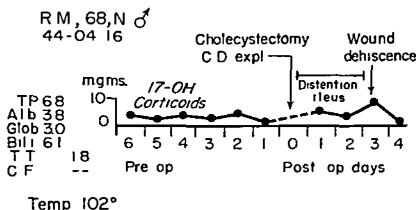



Fig 1 Urinary 17 OH corticoid excretion levels in a patient with gall stones and common duct obstruction The daily output prior to operation was low and there was only a slight adrenal response following operation and after repair of a wound dehiscence (Our figure for the first p o day in normal patients is 24 mg—see Fig 3)

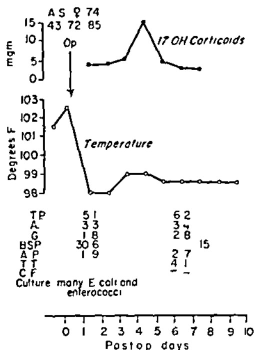



Fig 2 Urinary 17 OH Corticoid pattern in a critically ill patient following cholecystostomy for empyema of the gallbladder Increase in adrenal output was minimal and did not appear until the fourth postoperative day (The average output for normal patients on the first p o day was 24 mg—see Fig 3)

The 17 OH corticoid excretion pattern is unusually flat There was no increase in steroid output preoperatively despite the cholecystitis nor was there any significant increase in corticoid output following operation

A second example of corticoid patterns in jaundiced patients is shown in Figure 2 This 74 year old white woman was operated upon as an emergency because of an acute empyema of the gallbladder and a cholecystostomy was performed The patient tolerated the operation rather poorly having hypo tension and irregularities of pulse and respiration Following operation however she improved rapidly The temperature returned to normal her

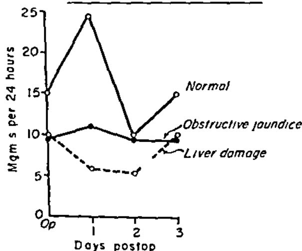



Fig 3 Comparison of average urinary 17 OH corticoid output following operation On the day of operation the normals averaged 15 mg and this increased to 24 mg on the first post operative day These outputs were 40 to 50 per cent greater than those for the obstructive jaundice group Patients with liver damage had even lower outputs

sensorium cleared and she was able to leave the hospital 11 days after operation with the cholecystostomy tube in place The steroid output showed no response to the stress of the empyema of the gallbladder or operation until the fourth postoperative day when a moderate rise was noted Following this the steroid levels promptly dropped to the previous levels

The average urinary 17 OH corticoid output on the day of operation for the normal patients in this study was 15 mg This rose to 24 mg on the first postoperative day was 10 mg for the second day and 15 mg on the third day In contrast the average output for jaundiced patients was 9 mg on the day of operation and 12, 9 and 9 mg on the first, second and third days respectively These data are shown in graphic form in Figure 3 which also includes corticoid outputs of 4 cirrhosis patients following operation The values for normal and jaundiced patients were subjected to an analysis of variance The difference between total hormone output during the 4 days was *significant at approximately the 5 per cent level* $(P = 0.05)$ *Analysis* was on only those patients with complete 4 day collections

DISCUSSION

**Normal Adrenal Response** Our findings on normal patients are consistent with currently held concepts of adrenal function Operative trauma stimulates a rapid increase in adrenal output which is reflected in a rise in urinary excretion on the day of operation Ordinarily the blood is cleared rather rapidly of excess amounts of steroid Fifty per cent of an intravenous dose of radioactive hydrocortisone leaves the blood within 3½ hr and 80 per cent appears in the urine during the first 24 hr[1] The systemic effects of operation seem to alter the metabolism of the steroids so that the peak urinary excretion comes during the 24 hr following the day of operation

**Adrenal Response in Jaundiced Patients** Our results in these patients appear to corroborate the hypothesis that hepatic dysfunction has a direct relation to urinary steroid excretion We have seen that even in normal patients operative stress retards urinary excretion due in part no doubt to *alteration in hepatic function The jaundiced patients in this series not only* had appreciably lower outputs on the day of operation but failed to show a significant rise on the first postoperative day and in general had a flat type of excretion curve

**Adrenal Response in Cirrhotic Patients** This group also had low corticoid output following operation The average output on the day of operation was 50 per cent less than normal patients The levels declined on the subsequent 2 days and then returned to the operative day figure on the third postoperative day (Fig 3) This tendency for delayed steroid excretion in patients with liver disease is additional evidence of the importance of the liver in steroid metabolism

**"Baseline" Levels** By this term we mean the average daily urinary output prior to operation The normal patients excreted 8 3 mg of 17 OH corticoids daily prior to operation which falls well within the normal range of one to 10 mg The jaundiced patients had an average daily excretion of 4 7 mg If this difference signifies depressed adrenal corticoid excretion in jaundiced patients then it may likewise imply an inability of the gland to respond to stress in normal fashion In cases of borderline operability and during postoperative complications this could be a critical deficiency

## SUMMARY

Urinary 17 OH corticoid levels in surgical patients have been measured before and after operation Three groups of patients tested were patients with normal livers those with obstructive jaundice and a few with cirrhosis We found a statistically significant difference in the hormone output after operation of these groups Compared to the normals the jaundiced patients excreted 10 per cent less on the day of operation and 50 per cent less on the first postoperative day They also had smaller outputs on the second and third days after operation The cirrhotic patients likewise had diminished urinary steroid levels Prior to operation the normal patients excreted an average of 8 3 mg daily The jaundiced patients excreted 4 7 mg daily a difference of 43 per cent These data support the concept of a direct relation between urinary 17 OH corticoid output and liver function They also imply that hepatic dysfunction may result in adrenal depression which under conditions of severe stress might require adrenal replacement therapy for survival



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## SOME STUDIES ON THE EFFICACY OF HETEROLOGOUS TETANUS ANTITOXIN*

A BURGESS VIAL AND WALTER S CALLAHAN

The common use of horse serum tetanus antitoxin and the frequent occurrence of individuals sensitized to horse serum lead to a high incidence of sensitivity reactions These foreign protein reactions are difficult enough to contend with but there arises the question of whether the foreign serum antitoxin actually protects the sensitized individual as well as it does the nonsensitized Certain work by other authors indicates that in diphtheria sensitized animals are not protected as well [1] and increasing doses of foreign tetanus antitoxin lead to increasingly rapid elimination of antitoxin [2]

In order to test the question further antibodies against human serum were developed in rabbits Tetanus antitoxin was developed in humans by repeated inoculations with tetanus toxoid After mixing these serums and incubating them for an hour at 37°C it was found that the supernate had lost more than one half of its antibody titer as judged by the Ramon flocculation test

*Table 1 Loss of Human Tetanus Antitoxin from Supernate Flocculation Tests*

| | | RABBIT ANTI HUMAN SERUM | NORMAL RABBIT SERUM | I S S |
|---|---|---|---|---|
| Human | Test 1 | 5.6 | 22.5 | 22.5 |
| Tetanus | Test 2 | 2.8 | 22.5 | 22.5 |
| Antitoxin | Test 3 | 5.6 | 11.2 | 22.5 |

It was demonstrated in another way too that mixing human tetanus antitoxin and rabbit antibodies against human serum removed tetanus antitoxin from the supernate the supernate was mixed with toxin and injected into mice and it was found that the supernate had lost most of its ability to neutralize the toxin

The human tetanus antitoxin must then have been precipitated in large part by the anti human serum It surprised the authors to find that the precipitated material still had some ability to protect mice against tetanus toxin although there was a great net loss of effective antibodies from supernate and precipitate That the precipitated antitoxin still had any antitoxin activity might be explained by assuming that part of the antitoxin was carried down as part of the large antigen antibody aggregate without the antitoxin being intimately involved in the reaction or the antitoxin mole

* University of Michigan Medical School Ann Arbor Michigan

cule may have different combining sites for the toxin and for the anti human serum

*Table 2 Loss of Human Tetanus Antitoxin Neutralization Tests*

| | RABBIT ANTI HUMAN SERUM SUPERNATE | PRECIPITATE | P.S.S |
|---|---|---|---|
| Human Tetanus Antitoxin | 19/25 | 20/25 | 0/25 |

Further tests were made mixing the human antitoxin and antibodies against the human serum After the mixture had been incubated at 37°C for an hour with constant shaking the whole material (i e bound and unbound antitoxin) was injected intramuscularly into mice the mice were then challenged with a given dose of toxin In one test for example 7/10 of the mice so treated died in 96 hr while only 2/10 of each of the control groups died

*Table 3 Loss of Human Tetanus Antitoxin Protection Tests (Mice Dead in 96 Hours)*

| | RABBIT ANTI HUMAN SERUM | NORMAL RABBIT SERUM | P.S.S |
|---|---|---|---|
| Human Tetanus Antitoxin | 7/10 | 2/10 | 2/10 |

This suggested that some of the antitoxin molecules were not just temporarily bound by the antibodies against the antitoxic serum but rather that the effect of the antitoxin was definitely reduced

When this last experiment was repeated but with intravenous administration of the mixture of human tetanus antitoxin and anti human serum the protective power of the antitoxin was again demonstrated to be reduced

It seemed pertinent to try to imitate the problem of the sensitized human by using animals administering heterologous antitoxin and then ascertaining the antitoxin levels periodically Guinea pigs were immunized with rabbit serum and then rabbit tetanus antitoxin was given the guinea pigs Controls consisted of unsensitized guinea pigs administered the same amounts of rabbit tetanus antitoxin The guinea pigs were then periodically bled and their antitoxin titers assayed Although the guinea pigs previously immunized with rabbit serum had initial antitoxin titers as high as the controls the titers fell more rapidly than did those of the controls

Judging from this test the guinea pigs previously immunized with rabbit serum were less well protected by the rabbit antitoxin

CONCLUSIONS AND SUMMARY

Heterologous tetanus antitoxin is less effective in animals immunized against the heterologous serum This was demonstrated by the fact that after an animal is immunized with heterologous serum his blood precipitates out

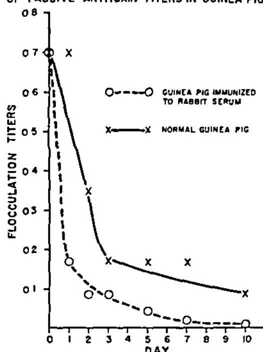



Fig 1

a large part of the heterologous antitoxin A part of this precipitated anti toxin can be recovered as effective antitoxin from the precipitate Administration of heterologous antitoxin to an animal immunized against the heterologous serum is followed by a more rapid loss of the antitoxin from the animal s circulation than from the serum of the control animal which has had no previous heterologous serum

This leads to the conclusion that human tetanus antitoxin would likely protect certain humans better than would animal antitoxin of similar titer It should be pointed out however that production of human tetanus anti toxin of high titer has not yet been accomplished

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# THE CONTROL OF POSTOPERATIVE WOUND INFECTIONS DUE TO ANTIBIOTIC-RESISTANT STAPHYLOCOCCUS AUREUS*

CHESTER W. HOWE

Recent publications[1, 2] and discussions with various surgeons indicate that in some surgical units epidemics of postoperative sepsis owing to *Micrococcus Pyogenes* (var. aureus) have reached serious proportions. Attention was called in a previous paper[3] to the increasing number of antibiotic-resistant strains of this organism being recovered from hospital patients and personnel throughout the world since the introduction of penicillin. Numerous surveys and bacteriophage typing studies indicate that the widespread use of penicillin has reduced the number of sensitive strains and allowed resistant staphylococci to flourish and become predominant. These resistant strains are then disseminated from patients to carriers and thence to other patients. This newly introduced phenomenon—namely, the dissemination of drug-resistant bacteria—has thus far been limited to hospitals and there is no evidence that it has affected the general population. To maintain a high incidence of resistant strains among carriers a large number of patients must be receiving the drug. This condition is fulfilled on a surgical service where penicillin is used heavily and constitutes one of the special situations where an increased number of staphylococcal outbreaks are being observed.

The purpose of this paper is to describe our experience with postoperative staphylococcal sepsis since 1949 and the measures we have taken to control it.

## METHOD

Accurate statistics are available on the incidence of postoperative wound sepsis following clean operations on the house and private surgical services of the Massachusetts Memorial Hospitals since 1949. All operations are classified as to their expectancy as regards sepsis at the time of operation.[3] Procedures where no contamination or infection is expected or justifiable, as for example herniorrhaphy or sympathectomy, and operations in which there is actual or potential contamination but in which wounds are normally expected to heal by primary intention, such as cholecystectomy or intestinal resection, are considered as clean. Infections are classified as major if they cause systemic manifestation, progressive invasion or destruction of tissue, prolonged hospitalization, or spontaneous or induced drainage of pus requiring therapy. Minor infections are those showing only localized inflammatory reaction in skin margins, stitch abscesses, or a trivial localized area of sepsis. The diagnosis of infection is made on a clinical basis in the closed postoperative wound. Infection is not diagnosed on the basis of a positive culture alone.

One large ward and the operating room floor were chosen as a sample cross section on which to perform a check of the hospital carrier rate of *Staphylococcus aureus*. On all persons appearing in these areas during a 24 hr. period, cultures of the nasopharynx and skin of the right hand were taken.

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* From the Departments of Surgery, Boston University School of Medicine and the Massachusetts Memorial Hospitals. Supported by a grant from the Trustees under the wills of Charles A. King and Marjorie King.

Floras were tested for sensitivity by placing a 1 unit penicillin disc* on blood agar plates freshly inoculated from the culture swabs Zones of inhibition 11 mm or less after 24 hr incubation at 37°C were considered resistant All others were considered sensitive If a large inoculum (solid sheet of growth) was not present the test was repeated with a heavy growth after subculture

All results were examined by Mr John Alman director of Statistical Research Services at Boston University Chi square analysis or the T test using the angular transformation were applied as indicated

### RESULTS

**Infection and Carrier Rates prior to 1953** Table 1 shows that the rates for major and minor infections combined was 1 09 per cent of the clean operations in 1949 but that there was a gradual stepwise statistically significant increase to 3 98 per cent in 1953 This occurred despite the widespread prophylactic use of antibiotics As in most hospitals the infection rate for house service cases was considerably higher than for private ones Analysis of the nutritional status age weight blood chemistry and hematological findings and type of surgery showed no significant difference in the clinical status between 1949 and 1953

Almost all the increase in infections over this 5 yr period was owing to major sepsis Table 2 shows the rates for major infections the ratio of infections caused by *Staphylococcus aureus* to those caused by other organisms *and the percentage of infections caused by this organism in the wounds that* were cultured The rate increased from 0 58 per cent in 1949 when 57 1 per cent of the infections were caused by *Staphylococcus aureus* to 2 85 per cent

*Table 1 Rates of Major and Minor Infections After Clean Surgery*

| | | HOUSE SERVICE | PRIVATE SERVICE | BOTH SERVICES |
|---|---|---|---|---|
| 1949 | No Operations | 401 | 796 | 1197 |
| | % Infections | 1 99% | 0 63% | 1 09% |
| 1950 | No Operations | 379 | 750 | 1129 |
| | % Infections | 3 16% | 1 06% | 1 77% |
| 1951 | No Operations | 366 | 843 | 1209 |
| | % Infections | 3 82% | 1 18% | 1 98% |
| 1952 | No Operations | 388 | 805 | 1193 |
| | % Infections | 5 15% | 1.24% | 2.51% |
| 1953 | No Operations | 429 | 800 | 1229 |
| | % Infections | 7.22% | 2.25% | 3 98% |
| *New Program Started* | | | | |
| 1954 | No Operations | 326 | 806 | 1132 |
| | % Infections | 5 21% | 1 61% | 2 65% |
| 1955 | No Operations | 321 | 845 | 1166 |
| | % Infections | 5.29% | 0 94% | 2 14% |

*Dia discs Commercial Solvents Corporation New York City

*Table 2 Major Infection Rate and Incidence of Staph Sepsis for Clean Surgery*

| | | MAJOR WOUND INFECTIONS | | | | | |
|---|---|---|---|---|---|---|---|
| | | | INFECTION RATE (PER CENT) | | | OWING TO | |
| YEAR | NO OF CLEAN OPERATIONS | NUMBER | HOUSE SERVICE | PRIVATE SERVICE | AVERAGES | STAPH RATIO | AUREUS PERCENT |
| 1949 | 1197 | 7 | 1.21 | 0.25 | 0.58 | 1 7 | 57.1 |
| 1950 | 1129 | 12 | 2.10 | 0.53 | 1.06 | 7 12 | 58.3 |
| 1951 | 1209 | 17 | 3.00 | 0.71 | 1.41 | 7 17 | 41.2 |
| 1952 | 1193 | 20 | 3.09 | 0.99 | 1.68 | 10 17 | 58.8 |
| 1953 | 1229 | [illegible] | 4.66 | 1.87 | [illegible] | 25 31 | 80.6 |
| *New Program Started* | | | | | | | |
| 1954 | 1132 | 18 | 3.37 | 0.86 | 1.59 | 15 17 | 88.2 |
| 1955 | 1165 | 18 | 3.42 | 0.82 | 1.54 | 9 16 | 56.2 |

*Table 3 Staphylococcal Nose and Skin Carrier Surveys*

| | NO OF CULTURES TAKEN | | | STAPH CARRIERS | | PENICILLIN RESISTANT STRAINS |
|---|---|---|---|---|---|---|
| DATE | PATIENTS | PERSONNEL | TOTAL | NUMBER | PERCENT | |
| Aug 1953 | 37 | 88 | 125 | 124 | 99.2% | 78.5% |
| Aug 1955 | [illegible] | 136 | 178 | 134 | 75.3% | 62.7% |

in 1953 when 80.6 per cent of them were owing to this organism Table 3 shows that 99.2 per cent of the sample of patients and personnel surveyed in August 1953 were *Staphylococcus aureus* carriers and that 78.5 of these were carrying penicillin resistant strains

**Corrective Measures** During the latter part of 1953 and early 1954 various measures to prevent cross contamination and the dissemination of antibiotic resistant bacteria were inaugurated as follows (1) The use of routine antibiotic prophylaxis was abandoned on the house service and largely curtailed on the private service Antibiotics were reserved for the treatment of established infections (2) Since the nasopharynx is the natural reservoir for staphylococci the use of two face masks (one over the other) during operations and the changing of these masks by the circulating nurse every 1½ hr was urged (3) A full 10 min scrub with a G 11 soap at least for the first case was urged (4) The established principles of meticulous skin preparation gentle surgery dry wounds and careful closure were emphasized (5) The use of face masks for doctors nurses and patients and of gloves during changing of dressings on the wards to help prevent the personnel from becoming carriers was instituted (6) Special septic sets containing instruments in an aluminum pan covered by a sterile double ply cloth wrapper were provided Soiled dressings were deposited in a large waxed paper bag placed in a closed container and incinerated daily At the conclusion of the dressing instruments gloves and other soiled material were placed in the pan which was wrapped and autoclaved before cleaning and definitive steril

ization (7) Strict aseptic technique as regards the dressing truck (8) Local isolation of infected wounds by impermeable occlusive dressings (isolation of patients proved impractical) (9) Prompt control of infected wounds by surgical drainage and debridement under antibiotic protection with early secondary closure where feasible

These measures were introduced over a *period of several months* and participation by the staff was generally good with some lapses by a few individuals

We are also aware of the possible role of fecal carriers blankets laundry and various housekeeping factors as sources of contamination No studies or measures directed toward these sources have been instituted as yet

**Infection and Carrier Rates after 1953** Table 1 shows that following the introduction of this program the overall infection rate dropped from 3 98 per cent in 1953 to 2 65 per cent in 1954 and to 2 11 per cent in 1955—a reduction by almost half which is highly significant statistically Table 2 shows that in 1955 the major infection rate fell to 1 51 per cent and in that year the proportion of infection owing to *Staphylococcus aureus* was down to 56 2 per cent

In August 1955 a survey using the patients and personnel in the same areas previously described was repeated using identical technique Table 3 compares these surveys and shows that the carrier rate was reduced from 99 2 to 75 3 per cent in the 2 year period and that the incidence of penicillin resistant strains in carriers fell from 78 5 to 62 7 per cent—both significant reductions

DISCUSSION

The staphylococcus has always been a common cause of wound sepsis and was found in 16 per cent of Meleney s clean wound infections over an 8 yr period in the pre antibiotic era Our rate of 80 6 per cent in 1953 appears therefore to be quite high and its reduction to 56 2 per cent in 1955 approaches pre antibiotic era levels Various studies[1] on the staphylococcal nasopharyngeal carrier rates of hospital patients and personnel before 1941 show ranges from 22 to 45 per cent so it would appear that our 1955 levels although improved are still abnormally elevated Many of the constituents of the normal respiratory flora are non pathogenic for wounds low in virulence or penicillin sensitive When there is a high hospital carrier rate of *Staphylococcus aureus* there is a greater chance for wound infection to develop because when contamination occurs there is an increased likelihood that it will be with this virulent organism rather than some of the non pathogens it has replaced

Examination of these data suggest that the increase in sepsis up to 1953 was related to an abnormally high carrier rate of penicillin resistant *Staphylococcus aureus* as a result of the widespread and prolonged use of penicillin and it is postulated that routine and extensive use of prophylactic antibiotics might therefore have an unfavorable effect on the postoperative infection rate

Improvement has followed the use of relatively simple and well known measures designed to prevent wound contamination and cross infection It appears likely that this improvement is related in some degree to the preventive measures outlined

The higher rate of infection in house service patients is our main problem and is currently being studied The inexperience of the resident staff is probably partly to blame but there are numerous other factors which may be contributory The house officer spends most of his time in the hospital environment does most of the dressings and has greater opportunity than the visiting surgeon to come in contact with resistant organisms and become a heavier carrier Private operations are likely to be shorter and the patients are apt to be in single rooms and hence less susceptible to cross contamination than those on the wards Host factors probably play an important role Since the development of medical insurance and Veterans Administration plans the majority of house patients are in the older age group Young good risk patients with uncomplicated surgical diseases are seldom seen on our wards at present

It is known that staphylococcal resistance will develop with other antibiotics than penicillin and that the development of resistance to an antibiotic is roughly in proportion to its use in a given area The resistance pattern of a bacterial species therefore may vary from time to time depending upon the antibiotic being used most extensively in a hospital

## SUMMARY AND CONCLUSIONS

1 Data on the clean wound infection rate at Massachusetts Memorial Hospitals for the period 1949–1955 are presented A high hospital carrier rate of penicillin resistant *Staphylococcus aureus* as a result of widespread use of this antibiotic is regarded as an important contributory factor toward the gradually increasing infection rate during 5 yr prior to 1954

2 During the last 2 years the carrier rate of this organism and the penicillin resistant strains in carriers have been significantly lowered and the postoperative wound infection rate has been reduced by almost half following institution of a relatively simple program for the prevention of cross infection the judicious use of antibiotics and meticulous wound management

3 The extensive prophylactic use of antibiotics in a hospital by virtue of the emergence and dissemination of resistant strains may intensify the problem of staphylococcal sepsis to such an extent as to contribute significantly toward an increase in the overall infection rate

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# EVALUATION OF PERITONEAL LAVAGE IN THE TREATMENT OF SEVERE DIFFUSE PERITONITIS

G Raymond Brown Jr and W Emory Burnett

The purpose of this experiment was to produce uniformly severe, diffuse *peritonitis in a laboratory animal, which compared with clinical human* peritonitis and to treat it by the various methods by which peritonitis is handled in the operating room

Guinea pigs were chosen as the subject of this experiment as they were readily available small relatively inexpensive, and less resistant to infection than rats or mice used by others They were obtained from one source and were approximately 6 wks of age Their average weight was 290 5 gm

Anesthesia consisted of veterinary pentobarbital sodium intra peritoneally Dosage was calculated at 30 mg /kg with the majority receiving 6 5 mg Duration of anesthesia was from 2 to 4 hr When an animal was reoperated upon the same day a second dose of 6 5 mg 1 hr after the first showed few complications The intra peritoneal route may be questioned but in none of the cases was there any evidence of contamination from the needle puncture Failure of anesthesia in several cases was thought to be due to intraluminal injection of the pentobarbital

A method of causing a uniform lethal peritonitis was sought one in which the animals would survive long enough that remedial therapy could be instituted Initially a 1 cm oval portion of the large cecum was excised but death occurred in 3 to 4 hr Then ellipses of various lengths were re moved from the colon The larger the ellipse the sooner death occurred It was decided that an incision in the colon might be better but these sealed off rapidly with more than 50 per cent of the animals surviving

Finally it was found that an ellipse 3 mm in length in the colon caused death in 17 of 20 guinea pigs and that 16 of these died in less than 24 hr All subsequent groups were therefore given peritonitis by this method

All guinea pigs were observed post operatively for a period of at least one week No special care was given except as noted below Death occurring post operatively within 2 hr automatically eliminated an animal from this series

Peritoneal cultures were taken in all groups Peritonitis was due to *E Coli B Subtilis* and *Streptococcus fecalis* (enterococcus) and sensitivity tests showed that these bacteria were susceptible to penicillin and streptomycin in combination Post mortem examinations were done routinely and bacterio logical studies were taken at that time An estimate of the amount of soiling present was made at the second operation

To show the results following variations in therapy 6 groups of 20 guinea pigs each were given peritonitis by the above method All groups were re operated upon 4 hr after the onset of peritonitis and the ellipse in the colon was repaired using #6 0 chromic catgut continuously and #5 0 silk inter rupted sutures Gross local contamination was removed and after specific therapy was carried out the abdominal wound was closed

**Group 1** was reoperated upon as described above The ellipse in the colon was repaired Local contamination was removed and the abdominal wall was closed No further therapy was given and 7 of the 20 animals died All peritoneal cultures were strongly positive

Group 2 was reoperated as above, the bowel was repaired similarly, local contamination was removed and prior to closing the peritoneum 5000 units of crystalline penicillin and 25 mg of streptomycin in 5 cc. of saline were instilled. Again 7 of the 20 animals died. Peritonitis was less severe by culture than in Group 1.

Group 3 was treated as before. However, the only therapy used was procaine penicillin G 30 000 units and streptomycin 50 mg intramuscularly for 2 days. Only 5 of the 20 animals died and cultures taken at post mortem were sterile in 3 and only a few bacteria were seen on the other 2.

Group 4 was prepared as previously with the exception that the peritoneal cavity was lavaged copiously with warm normal saline until gross contamination was removed. Five of the 20 animals died and cultures showed marked peritonitis at post mortem.

Group 5 was reoperated upon as before and the peritoneal cavity was lavaged with saline until gross contamination was removed and then relavaged with saline containing 100 000 units of crystalline penicillin and .5 gm of streptomycin per 500 cc. Six of the 20 animals died but of these 2 died on the third day of clinical and bacteriologically proven pneumonia.

Group 6 was prepared similarly and 4 hr. after the onset of the peritonitis they were reoperated, the ellipse in the colon was repaired and the peritoneal cavity was lavaged with copious amounts of warm saline containing 100 000 units of crystalline penicillin and .5 gm. of streptomycin per 500

*Table 1. Summary of Results*

| GROUP | TOTAL | DEAD | ALIVE | AMOUNT OF PERITONITIS BY CULTURE POST MORTEM | ESTIMATED AMOUNT OF SOILING |
|---|---|---|---|---|---|
| INITIAL (peritonitis only) | 20 | 17 | 3 | SEVERE | |
| 1 CLOSURE ONLY | 20 | 7 | 13 | SEVERE | ABOVE AVERAGE |
| 2 CLOSURE AND INSTILLATION P&S | 20 | 7 | 13 | MODERATE | ABOVE AVERAGE |
| 3 CLOSURE AND P&S IM | 20 | 5 | 15 | MILD | AVERAGE |
| 4 CLOSURE AND LAVAGE WITH NACL | 20 | 5 | 15 | SEVERE | AVERAGE |
| 5 CLOSURE AND LAVAGE WITH NACL AND P&S | 20 | 6* | 14 | MILD | AVERAGE |
| 6 CLOSURE AND LAVAGE WITH NACL AND P&S LAS IM | 20 | | 20 | NONE | AVERAGE |

(two died of pneumonia)

cc The abdominal cavity was then closed and intramuscular procaine penicillin 30 000 units and streptomycin 50 mg were given daily for 2 days None of the 20 animals died


SUMMARY

Severe diffuse peritonitis caused by removing an ellipse of colonic wall was found to be fatal in 17 of 20 guinea pigs

Six other groups of 20 guinea pigs each were prepared in this manner and were reoperated upon 1 hr later Each group was treated in a different manner

It was found that 1 Removal of the cause of the peritonitis alone reduces the fatality markedly 2 The amount of peritoneal soiling present at the time of operation is directly proportional to the mortality rate 3 Any further therapy besides removal of the cause of the peritonitis decreased the mortality 4 Local cleansing of the area of peritoneum involved and instillation of penicillin and streptomycin after removal of the cause of the peritonitis does not produce as good results as peritoneal lavage with saline without antibiotics and removal of the cause 5 The best results in the experiment were found in the group of animals in which the peritonitis was treated by peritoneal lavage with penicillin and streptomycin in saline and with that combination of antibiotics intramuscularly


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## THE HANDLING OF ABDOMINAL WOUNDS UNDER MASS CASUALTY CONDITIONS*

*An Experimental Study*


RUDOLPH A LIGHT MICHAEL G WEIDNER JR ROYCE E DAWSON DAVID P HALL AND JOHN L SAWYERS


In the event of a thermonuclear disaster Civil Defense and military personnel estimate that there will be no evacuation for 8 hours and no organized medical care for at least 12 hours following detonation In view of the overwhelming number of casualties medical personnel will be vastly inadequate and a policy has been formulated whereby several types of injuries will be set aside at the sorting station These will receive no therapy until those with greater chances of survival have received treatment These authorities justifiably feel that all surgery will necessarily be performed by inexperienced personnel under the supervision of a surgeon and that no more than 30 minutes can be allotted to each operation It is thought that this is insufficient time to perform life saving measures in the case of abdominal wounds [1] Therefore


From the S R Light Laboratory for Surgical Research and the Department of Surgery Vanderbilt University School of Medicine Nashville Tennessee This investigation supported by the Research and Development Division Office of The Surgeon General Department of the Army under Contract No DA 19 007 MD 66

these will be placed in the group to receive delayed therapy. No factual basis for this policy could be found and it was apparently formulated on the basis of Civil War experience.[1] This experiment is designed to assess the problem under simulated disaster conditions.

## METHOD

One hundred and eighty mongrel dogs were lightly anesthetized with 0.5 per cent intravenous thiopental sodium. One hundred and forty-four of these animals were shot with a .22 caliber target pistol using .22 caliber short ammunition with an estimated muzzle velocity of 1000 ft./sec. The animals were shot in three separate groups. They were placed on their sides over a sandbox and shot in the flank midway between the spinous processes and the midline of the abdomen. Groups 1 and 3 received wounds of entrance at the level of the 4th and Group 2 at the level of the 5th lumbar vertebra. Wounds of exit were directly opposite. In Group 1 (60 animals) there were 12 anesthetic controls that were not shot but were anesthetized each time the animals were operated upon. Groups 2 and 3 were composed of 60 animals each, with 12 animals in each group being anesthetized only at the time of shooting.

The animals were separated at the time of shooting into a group to be treated and a control group that was to receive no therapy. The 12 animals in each control group were placed in cages and observed. Animals in the group to be treated were placed in a large room with no food or water for a period of 12 hours. At the end of 12 hours the surviving animals were divided into 2 equal groups, 1 group being operated upon immediately, the survivors of the other group being operated upon at 36 and 60 hours. None of the animals were given any supportive therapy or antibiotic.

**Operation.** Animals were anesthetized with a continuous intravenous drip of 0.5 per cent thiopental sodium and the shaved abdomen was prepared with iodine and alcohol. The surgical teams were composed of 2 third-year medical students with no previous surgical experience. They donned caps and masks and scrubbed, using a one-brush technique. Sterile gloves, but no gowns, were worn. Each team had available 4 sterile towels for draping, 4 towel clips, 6 hemostats, 1 knife, 2 thumb forceps, 1 pair of scissors, 1 needle holder, 1 one-half curved Murphy and 1 Keith needle, 1 small retractor, 4-0 twisted silk and 20 4x4 gauze sponges. One qualified surgeon supervised and a circulating diener was available for each 5 teams. Operating time was limited to 30 minutes.

The abdomen was opened through a long vertical incision. A quick evaluation of the situation was made and the problem discussed with the supervising surgeon. Perforated bowel was closed if this could be done with 1 to 2 sutures. Large segments of necrotic bowel were excised, using mass ligation techniques, and the ends exteriorized. Where large perforations were present, that segment of bowel was exteriorized. Damaged spleens were removed, again using mass ligation technique. Bladder perforations were closed quickly if they were small or, if large, the bladder wall was sutured to the skin. The abdomen was closed using doubled 4-0 twisted silk as through-and-through sutures. All exteriorizations were made through stab wounds.

All animals except 3 in the non-operated group were autopsied. These unfortunately were lost.

*Table 1 Injuries by Site*

| SITE OF INJURY | GROUP 1 CONTROL | GROUP 1 OPERATION | GROUP 2 CONTROL | GROUP 2 OPERATION | GROUP 3 CONTROL | GROUP 3 OPERATION |
|---|---|---|---|---|---|---|
| Small bowel | 6 | 21 | 5 | 14 | 4 | 14 |
| Small bowel spleen | 5 | 8 | 1 | 2 | 7 | 8 |
| Small and large bowel | | 1 | | 3 | 1 | 3 |
| Small bowel bladder | | | | 3 | | 1 |
| Small bowel spleen bladder | | | | 1 | | |
| Small bowel mesentery | | | | | | 1 |
| Small bowel uterus | | 1 | | | | 2 |
| Uterus | | | | | | 1 |
| Uterus bladder | | | | | | 1 |
| Bladder mesentery | | | | 1 | | |
| Large bowel | | | | 1 | | |
| Bladder | 1 | 1 | 3 | 4 | | 4 |
| Broad ligament | | | | 1 | | |
| Omentum | | 1 | | 1 | | |
| Abdomen | | | | 5 | | 1 |
| Not specified | | | 3 | | | |
| TOTAL | 12 | 36 | 12 | 36 | 12 | 36 |

## RESULTS

**Sites of Injury** These are shown in Table 1 The animals that expired prior to 12 hours had predominantly bowel injuries and injuries to the spleen while those that survived more than 12 hours had a higher incidence of injuries to other organs such as bladder, broad ligament uterus, omentum and mesentery

**Survival** In the control group 26 dogs were living at the end of 12 hours with the ultimate recovery of 3 a survival rate of 12 per cent These 3 ultimate survivors had unspecified injuries In the group for operation 50 dogs survived 12 hours with the ultimate recovery of 12 a survival rate of 24 per cent In these survivors there were 5 bladder injuries 5 abdominal wall injuries 1 broad ligament injury and 1 omental injury These results are shown in Table 2 Five out of 30 animals operated upon at 12 hours 5 out of 9 at 36 hours and 2 out of 3 at 60 hours survived as shown in Table 3

**Mortality** The overall mortality in the first 12 hours in all animals that were shot was 47 per cent In the operative group no animal with a bowel perforation or uterine injury survived It is also interesting to note that no animal with perforated bowel survived as long as 36 hours without operation There were 2 deaths in the anesthesia controls which were used in Group 1 and there were 2 deaths in the remaining 21 animals giving an anesthetic mortality of 12 5 per cent

## DISCUSSION

Howard and DeBakey[2] reported a case fatality of 12 6 per cent in abdominal wounds receiving early treatment in Korea with a calculated increase in fatality of 0 5 per cent per hour delay of treatment Average operating time

*Table 2 Outcome of Injured Dogs that Survived 12 Hr, by Site of Injury and Handling*

| SITE OF INJURY | OPERATION GROUP 12 HR OPERATION | | OPERATION GROUP LATER OPERATION IF SURVIVING | | CONTROL GROUP NO OPERATION | |
|---|---|---|---|---|---|---|
| | 12 HR SURVIVAL | ULTIMATE SURVIVAL | 12 HR SURVIVAL | ULTIMATE SURVIVAL | 12 HR SURVIVAL | ULTIMATE SURVIVAL |
| Bowel injuries | 19 | | 10 | | 20 | |
| Uterus injuries | 1 | | 3 | | | |
| Bladder alone | 6 | 3 | 2 | 2 | 3 | |
| Other injuries | 4 | 2 | 5 | 5 | 3 | 3 |
| TOTAL | 30 | 5 | 20 | 7 | 26 | 3 |

*Table 3 Survival Figures*

| | CONTROL GROUP 12 HR SURVIVALS | OPERATION GROUP 12 HR SURVIVALS BY TIME OF OPERATION | | | | |
|---|---|---|---|---|---|---|
| | | 12 HR | 36 HR | 60 HR | NONE | TOTAL |
| Number of dogs | 26 | 30 | 9 | 3 | 8 | 50 |
| Number recovering | 3 | 5 | 5 | 2 | | 12 |
| % recovering | 12% | 17% | 56% | 67% | | 24% |

per abdominal case was 2 4 hours The conditions for evacuation and care of patients plus the inevitable presence of complicating associated injuries in the event of a thermonuclear disaster will without doubt increase the mortality rates which they cited However under survival conditions the operating times can be greatly lessened

The high first 12 hour mortality rate in this experiment is undoubtedly due to untreated hemorrhage and shock coupled with the fact that the very vascular spleen is disproportionately large and mobile in the dog To simulate the lack of competent anesthetists intravenous sodium thiopental drip anesthesia was selected and its control was by the supervising surgeons or circulating dieners This must have increased the mortality in the group receiving surgery However it must be noted that these were relatively clean wounds as compared to those one might see from flying debris The injured who survive immediate radiation flash or blast effects will in all likelihood receive wounds from secondary missiles at a velocity of approximately 800 ft /sec accompanied by vast quantities of dirt [1]

Although in this experiment ultimate survival 12 hours after receiving an abdominal wound is increased from 12 to 24 per cent by surgical intervention the justification for this procedure is conjectural Only surgically trained and aware personnel in the sorting stations can make the final decision under disaster conditions

CONCLUSIONS

1 Under the conditions of this experiment inexperienced personnel under supervision were able to perform adequate abdominal exploration and certain specified surgical procedures in 30 minutes

2 Such surgery does not improve the survival rate in bowel injuries but seems to improve the survival rate in other abdominal injuries

3 The high mortality in the first 12 hours coupled with the relatively slight improvement following operation confirms the justification of a policy of delayed evacuation of the abdominally wounded

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# THE USE OF NYLON TAFFETA IN THE REPAIR OF ABDOMINAL WALL DEFECTS*

ERIC M NANSON AND GAETANO T FIORICA

The majority of abdominal wall defects can be repaired with the use of adjacent tissue but when large defects exist it may be impossible to approximate the edges of normal tissue without marked tension In these cases it may be necessary to use implants of inert materials In the past autogenous material has been used but its limitations has led to increasing interest in the use of non living material many of these materials have now been used for a considerable length of time

The inert materials used fall into two groups metallic and plastic Metallic meshes have been most widely used but despite improvement in making these metals more biologically and chemically inert they are still liable to fracture due to their constant movement in living tissue The more pliable plastic materials appear to have overcome this inherent weakness of the metals

Of the various available plastics nylon has been the most widely used during the last few years Nylon is a material obtained by the synthesis of carbonium It has an homogeneous aspect and consistency Its specific weight is 1 14 and it liquifies at 250°C Its advantages are that it is biologically and chemically inert Being unaffected by minor changes in pH it can be sterilized easily by boiling or by autoclaving It appears to be durable and provides a framework within the tissues It has a high malleability and does not appear to fracture with constant flexion It is smooth radio translucent, inexpensive and readily available

The use of nylon mesh in the repair of herniae was first reported by Acquaviva and Bourret in 1944 [1, 2] Together with Cuture they used a fine mesh monofilament nylon sheet The individual filaments of the mesh were very thin (0 2 mm to 0 5 mm ) but were reported to be strong

Burmam[4] in 1948 published the results of 6 years work using nylon mesh in the treatment of very large umbilical herniae

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Corti in 1949 reported cases of large abdominal wall herniae in females which he treated with nylon mesh. One of his patients later had Caesarean section and the operative incisions were made through the nylon inlay. He reported that the incisions were not difficult to close and that the abdominal wall could be sewn in the usual manner. His results were satisfactory. Similarly in 1951 Aquino[3] reported his experiences using nylon in repairing 30 large hernia defects. He commented that the repair was simple, that the nylon appeared inert and that the procedure seemed valuable in places which would have been treated with difficulty by other methods.

Giraud, Vittori and Foucher[4] (1950) reported 6 cases. They stressed the importance of careful hemostasis and asepsis when using nylon inlays as 2 of their 6 cases became infected. Despite the infection the hernia inlays were intact. Stock in 1951 reported excellent results using wide mesh nylon in 10 inguinal hernias and 2 abdominal wall defects.

In the experimental field Testa[8] in 1951 used nylon to repair artificially created abdominal defects in animals. The created defects consisted of all muscular and fascial layers of the abdominal wall. He reported that nylon appeared to produce a minimal tissue reaction and that it appeared to be well tolerated by the tissues. His experiments were conducted on rabbits.

Moore and Siderys[7] in 1955 published the results of using nylon to repair similar defects in the anterior abdominal walls of dogs. In half the animals they used 3 layers of impervious nylon polyethylene sheeting as a graft. In some of the dogs they replaced the peritoneum with this inlay. The results of this maneuver were not satisfactory because of the high incidence of suture line dehiscence, fluid accumulation and marked tissue reaction. However they then went on to use fine mesh nylon in replacing various layers of the anterior abdominal wall including the peritoneum. They found the results much improved in that the nylon mesh was better tolerated than the impervious sheeting. They noted that the interstices of the grafts were invaded by fibroblasts thus incorporating the graft in the surrounding tissues.

Reviewing these reports it appears that nylon mesh may be the material of choice in surgical repair of large abdominal wall defects. The mesh can either be used to reinforce normal but weak tissue planes when the edges of the wound can be opposed or, if apposition is impossible, it can be used unsupported to substitute for the muscular and fascial layers and in some instances may be the only tissue between skin and peritoneum. Although the nylon appears to be inert and is well tolerated by the tissues it does cause a mild inflammatory reaction in the first few months. This goes on to a fibroblastic reaction which permeates the mesh. The resulting scar tissue strongly reinforces the nylon inlay.

## METHOD

In order to assess the validity of the above claims for the use of nylon mesh in hernial defects the following experiments were carried out in the Experimental Laboratory of the University Hospital.

Abdominal wall defects involving various layers of the abdominal wall approximately 8 cm by 4 cm were created in 32 healthy animals—16 dogs and 16 rabbits. These defects were then repaired as shown in Table I.

All animals were operated upon under intravenous nembutal anesthesia at a dose of 30 mgm./kg of body weight. In all cases the abdomen was shaved

*Table 1 Scheme of Nylon Taffeta Repair of Abdominal Wall Defects with Results*

| | | GRAFT REMOVED AT 3 MOS | AT 6 MOS | RESULTS |
|---|---|---|---|---|
| Ant Rectus Sheath only | Dogs | 5 | 3 | Satisfactory |
| Rectus Muscle with Ant & Post Rectus Sheath | Rabbits | 5 | 3 | Satisfactory |
| *Peritoneum only* | *Dogs* | *5* | *3* | *Satisfactory* |
| Peritoneum only | Rabbits | 5 | 3 | Satisfactory |

and disinfected with tincture of iodine 2½ per cent prior to the operation Postoperatively all animals were given antibiotics for 7 days by intramuscular injection In the majority of cases acromycin was used but in a few animals a penicillin streptomycin mixture was used

**Anterior Rectus Sheath Only** In 8 healthy dogs a rectangular piece of the anterior rectus sheath was excised The defect was then repaired with a rectangle of nylon taffeta a little smaller than the defect so that it was under tension The nylon graft was sutured to the fascial sheath using interrupted 3 0 silk The dogs made an uneventful postoperative recovery Moderate inflammatory reaction with edema was noted in all cases in the area of the operation for about 10 days This quickly settled but in 3 of the 8 dogs some thickening appeared to persist in this area In the other dogs the abdominal walls appeared quite normal After 3 months 5 of the dogs were sacrificed These included the 3 dogs which showed some residual inflammatory reaction The grafts were dissected out and subjected to histologic examination Macroscopically the graft was found to be incorporated in a sheet of fibrous tissue which was well united with the surrounding anterior rectus sheath In all 3 of the dogs that had shown clinically some persistent inflammatory reaction purulent exudate was found around the nylon but this had not interferred with the production of a strong sheet of fibrous tissue On microscopy the nylon mesh was surrounded by a narrow sheet of dense fibrous tissue which was infiltrated with a small number of lymphocytes and histiocytes (Fig 1) This infiltration was most marked immediately adjacent

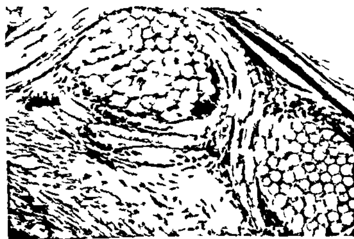


Fig 1 Microscopic appearance of nylon taffeta replacing anterior rectus sheath in a dog 3 mos after its insertion It shows dense fibrous tissue layers on either side of the nylon with lymphocytic infiltration between the threads

to the nylon filaments and in this neighborhood some foreign body giant cells were also noted

The other 3 dogs were sacrificed at the end of 6 months The microscopic findings were similar with the exception that the sheets of fibrous tissue were more dense

**Microscopic Description** The nylon is surrounded by a moderately thick layer of dense collagen which is poorly vascular and relatively acellular A light residual infiltration of lymphocytes and histiocytes lies next to the fabric and an occasional foreign body giant cell is also encountered

**Rectus Muscle and Anterior and Posterior Rectus Sheath** In this experiment rabbits were used In 8 animals a defect of about 8 cm by 4 cm was made in the anterior and posterior rectus sheath and rectus muscle The peritoneum was left intact as was the subcutaneous fascia A rectangular piece of nylon a little smaller than the defect was sutured in as already described Apart from a mild clinical inflammatory reaction during the first week these animals appeared well and their abdominal walls seemed normal during their whole postoperative period Five rabbits were sacrificed at the end of 3 months Macroscopically the graft was incorporated in a smooth layer of fibrous tissue The peritoneum underlying the graft was smooth and there were no adhesions noted between it and the intra abdominal organs On microscopy the same fibroblastic reaction with mild lymphocytic and histiocytic inflammatory reaction was seen There were rare giant cells immediately adjacent to some of the nylon threads but this was not nearly so marked as the foreign body reaction found around the sutures used to fix the graft to the tissue The remaining 3 rabbits were sacrificed at the end of 6 months The macroscopic findings were similar with the exception that the sheet of fibrous tissue incorporating the nylon mesh appeared to be a little denser The lymphocytic and histiocytic infiltration appeared to be less as if the inflammatory reaction were dying down

**Peritoneum only** Eight dogs and 8 rabbits were used A rectangular piece of peritoneum averaging 8 cm by 4 cm was dissected and cut off and a piece of nylon of the same size was substituted

The postoperative course was satisfactory The incisions healed well The nylon was removed after 3 months in 5 dogs and 5 rabbits Some adhesions were present between omentum and graft but they were easily separated The nylon was slightly wrinkled and covered by 2 layers of fibrous tissue The inner layer was like the peritoneum and in some animals it was difficult to recognize the demarkation line between the new and the old peritoneum In some animals there was a formation of new blood vessels in the new tissue The suture line in some animals was completely covered by serosal cells

On microscopic examination there was a thick layer of dense fibrosis on either side of the graft and there was also a moderate infiltration of lymphocytes and histiocytes with some foreign body giant cells

In 2 rabbits small foci of metastatic cartilage were found Some of these were calcified and some were converted into osseus tissue

In 3 dogs and 3 rabbits the grafts were removed after 6 months The findings were the same as those at 3 months

On microscopic examination the findings were the same as those at 3 months except that the inflammatory reaction was less marked In 1 rabbit

*[illegible] Nylon Taffeta Repair of Abdominal Wall Defects with Results*

| | | GRAFT REMOVED | | |
|---|---|---|---|---|
| | | AT 3 MOS | AT 6 MOS | RESULTS |
| [illegible] | Dogs | 5 | 3 | Satisfactory |
| [illegible] | Rabbits | 5 | 3 | Satisfactory |
| [illegible] | Dogs | 5 | 3 | Satisfactory |
| [illegible] | Rabbits | 5 | 3 | Satisfactory |

[illegible] with tincture of iodine 2½ per cent prior to the operation [illegible] all animals were given antibiotics for 7 days by intramuscu[illegible]. In the majority of cases achromycin was used but in a few ani[illegible] streptomycin mixture was used.

**[illegible] Sheath Only.** In 8 healthy dogs a rectangular piece of the [illegible] sheath was excised. The defect was then repaired with a [illegible] taffeta a little smaller than the defect so that it was under [illegible]. The nylon graft was sutured to the fascial sheath using interrupted [illegible]. The [illegible] made an uneventful postoperative recovery. Moderate in[illegible] with edema was noted in all cases in the area of the op[illegible] 10 days. This quickly settled, but in 3 of the 8 dogs some [illegible] appeared to persist in this area. In the other dogs the abdominal [illegible] quite normal. After 3 months 5 of the dogs were sacrificed [illegible] the 3 dogs which showed some residual inflammatory re[illegible] grafts were dissected out and subjected to histologic examina[illegible]. Macroscopically the graft was found to be incorporated in a sheet of [illegible] which was well united with the surrounding anterior rectus [illegible] of the dogs that had shown clinically some persistent inflam[illegible] purulent exudate was found around the nylon, but this had [illegible] with the production of a strong sheet of fibrous tissue. On [illegible] the nylon mesh was surrounded by a narrow sheet of dense fi[illegible] which was infiltrated with a small number of lymphocytes and [illegible] (Fig. 1). This infiltration was most marked immediately adjacent

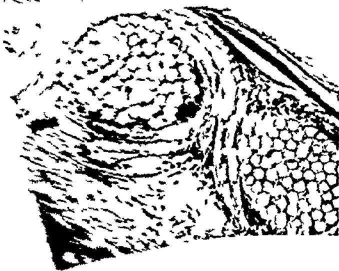


Fig. 1. Microscopic appearance of nylon taffeta replacing anterior rectus sheath in a dog, 3 mos. after its insertion. It shows dense fibrous tissue layers on either side of the nylon with lymphocytic infiltration between the threads.

to the nylon filaments and in this neighborhood some foreign body giant cells were also noted

The other 3 dogs were sacrificed at the end of 6 months The microscopic findings were similar with the exception that the sheets of fibrous tissue were more dense

**Microscopic Description** The nylon is surrounded by a moderately thick layer of dense collagen which is poorly vascular and relatively acellular A light residual infiltration of lymphocytes and histiocytes lies next to the fabric and an occasional foreign body giant cell is also encountered

**Rectus Muscle and Anterior and Posterior Rectus Sheath** In this experiment rabbits were used In 8 animals a defect of about 8 cm by 1 cm was made in the anterior and posterior rectus sheath and rectus muscle The peritoneum was left intact as was the subcutaneous fascia A rectangular piece of nylon a little smaller than the defect was sutured in as already described Apart from a mild clinical inflammatory reaction during the first week these animals appeared well and their abdominal walls seemed normal during their whole postoperative period Five rabbits were sacrificed at the end of 3 months Macroscopically the graft was incorporated in a smooth layer of fibrous tissue The peritoneum underlying the graft was smooth and there were no adhesions noted between it and the intra abdominal organs On microscopy the same fibroblastic reaction with mild lymphocytic and histiocytic inflammatory reaction was seen There were rare giant cells immediately adjacent to some of the nylon threads but this was not nearly so marked as the foreign body reaction found around the sutures used to fix the graft to the tissue The remaining 3 rabbits were sacrificed at the end of 6 months The macroscopic findings were similar with the exception that the sheet of fibrous tissue incorporating the nylon mesh appeared to be a little denser The lymphocytic and histiocytic infiltration appeared to be less as if the inflammatory reaction were dying down

**Peritoneum only** Eight dogs and 8 rabbits were used A rectangular piece of peritoneum averaging 8 cm by 1 cm was dissected and cut off and a piece of nylon of the same size was substituted

The postoperative course was satisfactory The incisions healed well The nylon was removed after 3 months in 5 dogs and 5 rabbits Some adhesions were present between omentum and graft but they were easily separated The nylon was slightly wrinkled and covered by 2 layers of fibrous tissue The inner layer was like the peritoneum and in some animals it was difficult to recognize the demarkation line between the new and the old peritoneum In some animals there was a formation of new blood vessels in the new tissue The suture line in some animals was completely covered by serosal cells

On microscopic examination there was a thick layer of dense fibrosis on either side of the graft and there was also a moderate infiltration of lymphocytes and histiocytes with some foreign body giant cells

In 2 rabbits small foci of metastatic cartilage were found Some of these were calcified and some were converted into osseus tissue

In 3 dogs and 3 rabbits the grafts were removed after 6 months The findings were the same as those at 3 months

On microscopic examination the findings were the same as those at 3 months except that the inflammatory reaction was less marked In 1 rabbit

the nylon graft was heavily calcified The calcium was deposited in the fibrous tissue immediately adjacent to the nylon Here it formed large solid plaques but on occasion there was a network of calcium in the interstices between the individual nylon filaments

## CONCLUSIONS

Nylon taffeta is a satisfactory material for use in replacing defects in the muscle aponeurosis and peritoneal layers of the abdominal wall

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# EVALUATION OF DACRON SUTURE MATERIAL FOR GENERAL SURGERY*

JOSEPH K NARAT, JOSEPH P CANGELOSI AND JOHN V BELMONTE

Evaluation of dacron suture material† was undertaken to continue our studies on synthetic suture materials [1, 2] Dacron is a polymer, namely a condensation product of ethylene glycol and dimethyl terephthalate Dacron polyester fiber is not derived from tar nor does it contain tar

The tensile strength of various gauges of non sterile as well as autoclaved dacron on straight pull dry knot and wet knot pull was compared with that of cotton and silk Some of the results recorded in Table 1 show that dacron is stronger than cotton or silk For instance in one series of experiments the dry knot tensile strength of dacron size 1 0 was 57 per cent greater than that of the corresponding gauge of cotton dacron size 3 0 was 61 per cent stronger and dacron size 2 0 was 27 per cent stronger than the same sizes of cotton The tensile strength of dacron was also slightly greater than that of silk It was found that the tensile strength of wet dacron is identical with that of dry material

Determination of the frictional coefficient an important factor in the knot holding property of suture materials gave the following results dacron 0 58 silk 0 52 nylon 0 47 and cotton 0 22 In other words the fractional co efficient of dacron was 2 6 times as great as that of cotton

*From the Department of Surgery University of Illinois College of Medicine Chicago Illinois
†Furnished by Ethicon Inc New Brunswick N J

*Table 1 Comparison of Sterile Dacron Cotton and Silk*

| | SIZE | DACRON | COTTON | SILK | REMARKS |
|---|---|---|---|---|---|
| | | | | | Dacron stronger than cotton |
| Knot Tensile | 2 0 | 4 40 | 3 51 | 1 1 | 27% |
| Strength | 3 0 | 3.31 | 2 05 | 2 8 | 61% |
| in lb | 4 0 | 2.23 | 1 42 | 2 1 | 57% |
| Frictional Coefficient | | 0 58 | 0.22 | 0.52 | Frict coeff of Dacron 2 6 times as great as that of cotton |
| Waxed | | no | yes | yes | |
| Electron Sterilization | | applic able | not applic able | not applic able | |
| Weakened by Autoclaving | | no | yes | yes | |

Contrary to cotton repeated autoclaving does not reduce the tensile strength of dacron For example one batch of dacron autoclaved 7 times did not show any diminution in tensile strength

Dacron thread does not fry The material is furnished unwaxed while commercial cotton contains wax This is of importance because acute tissue reactions to waxes derived from spool cotton followed by formation of gran ulomas have been described by Rosenberg *et al* [3]

Electron sterilization of suture materials which is rapidly gaining popu larity is applicable to dacron but not to cotton which disintegrates under the influence of rays There is no loss in tensile strength of dacron sutures when irradiated at 3 million reps *

To study *tissue reaction to dacron strands of dacron cotton* silk and catgut were implanted into the abdominal wall of rabbits and biopsies were obtained at various periods after the procedures The histologic examina tion showed the intensity of tissue reaction to dacron to be approximately equal to that following insertion of other suture materials

To test the behavior of dacron *in vivo* the following experiment was de vised Under aseptic conditions the abdominal wall of rabbits was incised and the peritoneum and fascia were closed with either interrupted or contin uous sutures of various gauges of dacron cotton silk or linen respectively At various periods after this procedure the animals were sacrificed the entire *abdominal* wall was excised and placed like a diaphragm over a round wooden box which had 2 adapters one connected with a sphygmomanometer and the other with a compressed air outlet This arrangement made it possible to in flate the interior of the box to 250 mm Hg which is the highest intra abdomi nal pressure recorded in man The inflation and deflation were repeated 100 times to imitate conditions created by gagging sneezing vomiting coughing or defecation Numerous experiments showed that during the critical period namely from the third to the fifth postoperative day (when the strength of the wound is at its lowest ) dacron gauge 2 0 or 3 0 was able to withstand a pressure of from 200 to 250 mm Hg

* Abbreviation of roentgen equivalent physical unit

We have used dacron in 89 operations such as thyroidectomy, cholecystectomy, intestinal anastomosis and herniorrhaphy without any untoward results. No extrusion or formation of granuloma occurred.

SUMMARY

The tensile strength and frictional coefficient of dacron are superior to those of cotton and silk. Dacron does not fray, is not affected by repeated autoclaving, contains no wax, and is well tolerated by tissues. Therefore the statement seems justified that dacron deserves an important place among non-absorbent suture materials.

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# THE REPAIR OF DEFECTS IN THE DIAPHRAGM OF DOGS USING SHEETS OF IVALON SPONGE AND FRESH HOMOLOGOUS GRAFTS OF FASCIA LATA*

IRVING G. PESEK, ARNE E. SCHAIRER AND JOHN L. KEELEY

Prior work in the repair of defects in the diaphragm of dogs using fresh homologous grafts of fascia lata stimulated a study in which sheets of polyvinyl sponge (Ivalon) were used to correct similar defects. This synthetic material is made from foamed polyvinyl alcohol hardened with formaldehyde. It is white and its cut surface has the porous appearance of bread. When dry it is somewhat firm and rigid but becomes soft and elastic when moistened. It has a remarkable affinity for water and except for slight shrinkage is unchanged by temperatures reaching 120°C. Further, polyvinyl sponge appeared to possess the desirable qualities for a synthetic substance useful in the repair of hernia. Grindlay and Waugh[1] on the basis of their experience with polyvinyl sponge suggested that the affinity of polyvinyl sponge for water gave it a "kinship" with living tissue in which cells exist in an environment of water with fibrous tissue eventually invading and surrounding the polyvinyl sponge, thus in a sense the inert becomes living. Polyvinyl sponge has been used in the successful repair of created defects in the abdominal wall of dogs and as subcutaneous protheses.[2, 3, 4] Under these conditions it is possible that the abdominal wall or the skin and underlying

* From the Department of Surgery, Stritch School of Medicine of Loyola University, Chicago, Illinois.

tissue afforded a supportive framework and perhaps more effective contact with other tissues from which fibroblasts would grow than in the diaphragm where the insert would be subjected to more deforming forces and therefore tested more vigorously

METHOD

Twenty dogs weighing from 7 2 to 12 3 kg were used Anesthesia was provided by intraperitoneal nembutal and respiration was maintained by insufflation of room air through an endotracheal tube In all instances the dogs received intravenous dextrose during the operative procedure The left hemithorax was entered through a laterally placed incision in the lower chest wall Approximately ¼ to ½ of the muscular portion of the left hemidiaphragm was excised Sheets of Ivalon 3 to 4 mm thick previously sterilized by boiling in tap water for 30 min were sutured to the peritoneal side of the diaphragmatic defect The sponge implant was secured by means of interrupted and continuous sutures of 4 0 cotton achieving an imbrication of 1 cm The chest wall was then closed in layers with cotton sutures and without drains No intrapleural antibiotics were used however the dogs received daily injections of 300 000 units of penicillin for the first 3 days after operation Later in the study roentgenographic examinations were made after pneumoperitoneum was induced for better visualization of the diaphragmatic contours Animals were sacrificed at intervals of 1½ 2 4 6 and 12 mo at which time gross and microscopic studies were made

RESULTS

In the immediate postoperative period apparently due to anesthesia 2 dogs were lost in the first 24 hr The rest of the animals began to be active about 3 to 5 days after operation The x ray studies made at intervals before sacrifice showed normal diaphragmatic contours and lung fields (Fig 1) At autopsy there were firm adhesions between the sponge insert and the lung stomach liver and omentum In no instances were any collections of encapsulated fluid noted about the sponge In the animals sacrificed after 12 mo *the sponge insert was firm and it was difficult to identify* upon viewing the gross specimen (Fig 2)

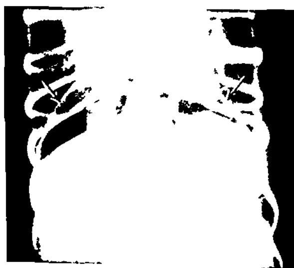


Fig 1 Roentgenogram showing diaphragmatic contour in dog 6 mos after implant of sponge

We have used dacron in 89 operations such as thyroidectomy cholecystectomy intestinal anastomosis and herniorrhaphy without any untoward results No extrusion or formation of granuloma occurred

## SUMMARY

The tensile strength and frictional coefficient of dacron are superior to those of cotton and silk Dacron does not fray, is not affected by repeated autoclaving contains no wax and is well tolerated by tissues Therefore the statement seems justified that dacron deserves an important place among non absorbent suture materials

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Prior work in the repair of defects in the diaphragm of dogs using fresh homologous grafts of fascia lata stimulated a study in which sheets of poly vinyl sponge (Ivalon) were used to correct similar defects This synthetic material is made from foamed polyvinyl alcohol hardened with formaldehyde It is white and its cut surface has the porous appearance of bread When dry it is somewhat firm and rigid but becomes soft and elastic when moistened It has a remarkable affinity for water and except for slight shrinkage is unchanged by temperatures reaching 120°C Further, polyvinyl sponge appeared to possess the desirable qualities for a synthetic substance useful in the repair of hernia Grindlay and Waugh[1] on the basis of their experience with polyvinyl sponge suggested that the affinity of polyvinyl sponge for water gave it a kinship with living tissue in which cells exist in an environment of water with fibrous tissue eventually invading and surrounding the polyvinyl sponge thus in a sense the inert becomes living Polyvinyl sponge has been used in the successful repair of created defects in the abdominal wall of dogs and as subcutaneous protheses[2 3 4] Under these conditions it is possible that the abdominal wall or the skin and underlying

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Fig 3 Photograph showing diaphragm of dog after repair with homologous fascia lata

was replaced by a layer resembling granulation tissue Finally the graft was represented by a sheet of dense fibrous tissue which appeared to be the supporting structure Only fragments of the original graft could be identified microscopically It appears that homologous fascia lata grafts are satisfactory in the repair of diaphragmatic defects during the period of observation However they do not survive intact as autogenous fascia lata grafts are reported to do when used in the same manner but observed for a shorter period of time[5]

CONCLUSIONS

In 2 series of animals approximately 60 per cent of the central portion of the left diaphragm was excised Repair of these defects was accomplished by use of Ivalon sheet in one series and fresh homologous fascia lata grafts in the other Both materials appear to be effective in the repair of defects thus created and observed for periods up to 12 mo

From the preliminary examinations of the microscopic sections in these studies both polyvinyl sponge and fascia lata form a framework about which a sheet of fibrous tissue grows The sponge appears to undergo fragmentation while the fascia lata appears to undergo absorption and replacement

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Fig 2 Photograph showing diaphragm of dog removed 12 mos after implant of sponge

Microscopically the findings which we noted were similar to those previously reported by Grindlay and Waugh[1] The sections showed spaces irregular in size and arrangement filled with a faint blue material after staining with hematoxylin and eosin Higher magnification disclosed that these pale blue areas had a porous bubble like appearance Interspersed between these spaces were infiltrations of collagen fibers containing spaces lined by a single layer of cells which may be vascular endothelium as has been suggested or may represent atypical flattened fibroblasts In the specimens studied 1½ and 2 mo after implantation there was a marked but loose fibrous ingrowth around the sponge and no inflammatory reaction was noted At the 4 mo interval the sponge appeared to be somewhat more compact and the connective tissue was more dense but sparsely cellular Later at the 6 mo interval the sponge trabeculae appear fragmented with very abundant foreign body giant cell reaction At the 12 mo interval the trabecular fragmentation was still noted with considerably less trabeculae present and more acellular fibers It becomes apparent then that for some reason a foreign body giant cell reaction is provoked sometime during the interval of 4 to 6 mo after implantation We do not know the significance of this reaction but subsequent studies may show that this represents a critical period in the assimilation of the graft We feel that this should stimulate a further study of this giant cell reaction

In another series of experiments under essentially the same conditions of anesthesia exposure and technique as described in the use of Ivalon sheets but without antibiotics fresh homologous grafts of fascia lata obtained from a dog s thigh were used to repair diaphragmatic defects of similar size All animals survived and were observed for periods up to 12 mo X ray studies showed no disturbance in the contour of the diaphragm and no evidence of herniation The animals were likewise sacrificed at intervals and gross examination disclosed completely satisfactory repair of the defect in 13 dogs (Fig 3) In 1 dog the graft was thin and eventration involving about 60 per cent of it occurred Multiple microscopic sections of the grafts taken at necropsy showed an early inflammatory reaction on both surfaces of the entire graft as indicated by edema and infiltration with polymorphonuclear leukocytes lymphocytes and plasma cells Later the inflammatory reaction

In this first phase of the study, the spontaneous respiratory efforts of the patient on the ventilator were abolished by deepening the level of anesthesia; in subsequent studies this will be achieved by hyperventilating the individual.

Ether was the basic anesthetic agent employed in almost all cases.

## RESULTS

To date 30 patients have been studied, 15 with the ventilator and 15 without. The averaged values for the 2 groups are shown in Table 1.

**Arterial Oxygen Saturation** The average blood oxygen saturation was depressed in both groups immediately prior to operation, a finding which later proved to be due largely to the effect of relatively heavy pre-anesthetic medication which included morphine or demerol and a barbiturate. Samples taken in subsequent patients prior to the pre-anesthetic medication, or when only atropine was given, were generally within normal limits. This moderately diminished preoperative arterial oxygen saturation was accompanied, as noted below, by a mild increase in total carbon dioxide content of arterial blood. However, this increase was not sufficient to alter the blood pH to a degree detectible with the pH meter.

In general, the degree of arterial oxygen saturation, while somewhat below normal in both groups, was perhaps more uniform in the "respirator" group; yet this average difference was not great. This was so even though the "respirator" group was carried at a depth of anesthesia sufficient to halt all respiratory efforts on the part of the patient. Isolated instances of hypoxia occurred in both groups, but it was only in the group in which the Jefferson ventilator was not used that severe cardiac depression due to poor ventilation occurred. Virtual cardiac arrest intervened in 1 patient and profound shock in the other—both of whom responded to vigorous and rapid manual compression of the gas bag. Of course, hypoxia may result from oligemic shock as well as other causes.

**Total Arterial $CO_2$ Content** Whereas during the control period the average total carbon dioxide content of arterial blood tended to be elevated (likely due to the heavy pre-anesthetic medication in the earlier patients), there was a gradual average decline in this value during the period of anes-

*Table 1. Cumulative Average Values (Arterial Blood): 15 Patients with Jefferson Ventilator and 15 Without*

| Ventilator | | C | After Endotracheal Tube: 1 (10 min) | 2 (25 min) | 3 (40 min) | 4 (70 min) | 5 (130 min) | Recovery Room | Day After |
|---|---|---|---|---|---|---|---|---|---|
| With | pH | 7.48 | 7.45 | 7.44 | 7.49 | 7.50 | 7.54 | 7.47 | 7.49 |
| Without | pH | 7.44 | 7.43 | 7.41 | 7.42 | 7.42 | 7.44 | 7.37 | 7.46 |
| With | Total CO Content | 48.55 | 48.07 | 47.12 | 47.21 | 43.97 | 42.76 | 46.76 | 49.47 |
| Without | Total CO Content | 46.24 | 44.5[illegible] | 43.87 | 47.81 | 47.36 | 42.74 | 47.70 | 47.01 |
| With | Per cent Oxygen Saturation | 81.12 | 91.53 | 90.82 | 85.21 | 84.46 | 84.2[illegible] | 82.90 | 82.74 |
| Without | Per cent Oxygen Saturation | 87.1 | 93.9 | 91.8 | 97.8 | 91.4 | 82.3 | 88.0 | 86.5 |

* The relatively depressed control levels were found to be due in part to the effect of the pre-anesthetic medication.

# *Anesthesia and Pulmonary Surgery*

## ANESTHESIA RESEARCH I USUAL vs MECHANICAL METHOD OF INFLATION*

JAMES D HARDY JOHN O DAMPEER JR M DON TURNER AND GLACE BITTENBENDER

Cardiac arrest due to defective anesthetic management constitutes a major source of operative mortality Moreover, inadequate pulmonary ventilation ranks high as a specific example of defective anesthesia When the minute gas exchange is below a critical level for the patient in question the potentially lethal conditions of carbon dioxide retention with acidosis and of anoxia result Many workers have shown (and countless surgeons have suspected from the color of the blood) that anoxemia and hypercapnia usually have existed for some time before the heart succumbs One facet of the problem would appear to be the need for an increased awareness on the part of many anesthetists as to what adequate ventilation entails for anesthetists with sound basic training in cardio respiratory physiology rarely allow the patient to become anoxic or hypercapneic This is perhaps but another way of stating that there exists a shortage of well trained anesthetists However it occurred to us as it had to others that a mechanical device which could be set to ventilate the patient at a definite minute rate and volume might enhance the efficiency of pulmonary ventilation if properly employed For this reason we have conducted a clinical comparison between usual intermittent manual assistance and the Jefferson ventilator

### METHOD

Prior to operation a specially prepared No 17 needle was inserted into a femoral artery Control and subsequent intermittent arterial blood samples were drawn throughout the period of anesthesia and during recovery therefrom The following day a final arterial blood sample was drawn Blood oxygen and total carbon dioxide analyses were made with the Van Slyke manometric blood gas apparatus and blood pH measurements with a Beckman pH meter

Mechanical inflation was used in approximately one half the patients studied As soon as the endotracheal tube had been inserted the ventilator was switched into the system to inflate the lungs at a fixed rate and volume employing both positive and negative phases The degree of inflation was regulated by direct inspection of the lung in patients having open chest operations and by clinical judgment in those having abdominal procedures The clinical judgment of the anesthetist was important even when using the machine therefore since the ventilator was regulated by this individual

*Department of Surgery University of Mississippi Medical Center Jackson Mississippi
This work performed under Army Contract No DA 49 007 MD 627 Office of the Surgeon General Department of the Army
With the technical assistance of Virginia Ward and Thelma Carter

# THE EFFECT OF ETHER ANESTHESIA ON PYRUVATE METABOLISM*

WILLIAM R DRUCKER CHRISTINE COSTLEY ROBERT STULTS
MAX MILLER JAMES W CRAIG AND HIRAM WOODWARD

Reynoso[1] reported in 1853 that gylcosuria followed ether anesthesia in man It was not until after the turn of the century however that a report was made relating an increase in blood sugar to ether anesthesia [2] In the following 50 years considerable attention was directed toward the disturbance in carbohydrate metabolism associated with anesthesia because of the possibility that inhibition of cellular carbohydrate metabolism is responsible for ether narcosis This work has been reviewed by Gerard [3]

Previous studies done in our laboratory concerned the effect of ether anesthesia in man on the metabolism of two hexoses glucose and fructose [4] It was found that ether caused a marked disturbance in the tolerance for glucose but relatively little disturbance in the tolerance for fructose The result was interpreted as indicating that ether inhibits specific enzymatic processes

The next step in the study of the effect of ether on carbohydrate metabolism in humans was to determine the effect of ether on pyruvate metabolism This seemed logical because pyruvic acid is the final product in the anaerobic scheme of glycolysis and it is converted into acetyl CoA which supplies fuel for the citric acid energy producing cycle Also many of the studies on tissue slices have indicated that narcotics may act on the metabolism of pyruvate

## METHOD

Five patients hospitalized for rehabilitation at Highland View Cuyahoga County Hospital volunteered for this study Each patient received 2 pyruvate tolerance tests 1 a day or 2 prior to anesthesia as a control and 1 during the administration of anesthesia Supplementary vitamin B complex was given for a few days prior to the study in doses that were 10 to 60 times the normal requirement The subjects were fasted and required to remain in bed on the day of the tests Blood samples drawn through a Cournand needle were obtained prior to the start of anesthesia and subsequently every 30 min during the 90 min of anesthesia and the 2 hr following the cessation of ether administration Anesthesia was induced by the intravenous administration of 150 to 300 mg of thiopental sodium Ether anesthesia was started with a mixture of nitrous oxide oxygen and ether and maintained with endotracheal oxygen ether Thiopental sodium was used to minimize the excitement frequently encountered during the induction of ether narcosis All anesthetics were given by the same person (C C) Thirty minutes after surgical anesthesia was obtained a specimen of blood was drawn and an infusion of sodium pyruvate was started The infusion was given at a con

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With the technical assistance of Nancy Hofmann Helen Bassett Naomi Nickerson and Eloe Mirek

The authors express their appreciation to Warren Strauss for his assistance with the illustrations

thesia, whether the Jefferson ventilator was or was not used These values regained control or normal levels while the patients were under post anesthetic observation in the recovery ward Thus it would appear from the data that on the average a degree of hyperventilation was produced in both groups

**Arterial pH** The average values for the 2 groups of patients were similar but there was some divergence of the pH toward the alkaline side in the ventilator group and toward the acid side in the usual group In isolated patients particularly in the usual group significant acidosis was encountered

DISCUSSION

By averaging the values in each group one achieves generalization and a certain balance in the evaluation of the data satisfactory ventilation *can* be achieved by either method At the same time, highly significant exceptions—the isolated patients in whom anesthesia was not satisfactory—are obscured by averages Let it suffice to state here that instances of seriously defective anesthesia occurred primarily in the group in which the ventilator was not employed Moreover as the study progressed and the purpose of the arterial blood sampling became known to the personnel, there was a tendency for the nurse anesthetists to utilize more manual assistance and to diminish the incidence of defective ventilation in the usual group

SUMMARY AND CONCLUSIONS

1 Thirty patients undergoing general anesthesia have been studied with serial determinations of the oxygen saturation, pH and total $CO_2$ content of arterial blood In approximately one half, the usual spontaneous respiration with or without indicated manual assistance was employed in the other half, the Jefferson ventilator was switched into the system as soon as the endotracheal tube was in place Ether was the basic anesthetic agent in virtually all cases

2 The use of an atropine morphine barbiturate pre anesthetic medication rendered pre anesthetic control levels of arterial oxygen saturation somewhat below normal in the earlier patients studied, control arterial total $CO_2$ content was mildly increased In subsequent subjects the control sample was drawn before the pre anesthetic medication had been given and furthermore the dosage of such medication was reduced

3 In general the arterial pH total $CO_2$ content and oxygen saturation were comparable in both groups However serious and clinically obvious anesthetic difficulties were not encountered in the group in which the Jefferson ventilator was used whereas 2 such instances occurred in the group in which it was not used No ill effects were observed from the ventilator

4 It is concluded that while efficient management can be conducted without an artificial ventilator, the judicious use of such an aid adds to the safety factor in avoiding hypoventilation

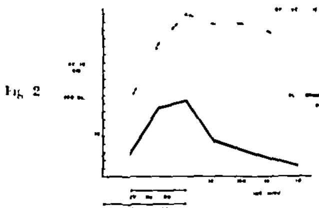


Fig 2

out ether are illustrated in Figure 2 The lactic acid rise during the first 30 min of pyruvate infusion averaged 5 5 mg /100 ml for the control tests and 14 5 mg /100 ml for the anesthesia tests The differences between the 2 tests became progressively more pronounced with time Two hours after discontinuation of the infusion and anesthesia the lactic acid level remained 12 6 mg /100 ml above the fasting level and 5 6 mg /100 ml above the level obtained at the time the infusion was started (30 min after the start of anesthesia)

**Glucose** The mean rise of fasting blood glucose during the first 30 min of anesthesia was 39 mg /100 ml When the pyruvate infusion was started the glucose level fell in both the control and anesthesia tests

DISCUSSION

Pyruvic acid stands at the cross roads of protein fat and carbohydrate metabolism (Fig 3) Pyruvate may be converted into protein, fat glycogen, lactate oxaloacetate or oxidized *via* acetyl CoA into the citric acid cycle to yield energy There may also be pathways of pyruvate metabolism presently unknown It is not possible to discern from the present study which of these many pathways of pyruvic acid metabolism is blocked by ether anesthesia Considerable work on tissue slices however suggests that oxidative decarboxylation of pyruvate is blocked probably at some point in the cytochrome system

Decarboxylation of pyruvic acid requires 4 interdependent factors 1 thi

PATHWAYS OF PYRUVATE METABOLISM

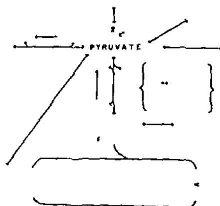



Fig 3

stant rate with an infusion pump for 1 hr at the end of which time both the infusion and the ether were discontinued Capillary oxygen content was followed at frequent intervals by an ear oximeter Samples of arterial blood were drawn for analysis of oxygen saturation prior to the start of anesthesia and 30 and 90 min after surgical anesthesia was reached An electrocardiogram was obtained on the day prior to the test and frequent recordings were made during the period of narcosis

Crystalline sodium pyruvate* dissolved in pyrogen free water† was filtered through a Seitz Filter at 10 mm Hg suction pressure The solution was then diluted to contain 16 5 gm of sodium pyruvate/L of solution (13 2 gm pyruvic acid/L) The pH was adjusted to 7 40 by the addition of 0 45 ml of 0 1 N HCL/L Each subject was given 9 09 ml (0 12 gm )/kg of body weight over a 1 hr period This dose was found by us to produce a rise in blood pyruvic acid of 2 3 mg /100 ml in non diabetic hospitalized individuals All solutions were prepared within 2 days of use and were refrigerated until the time of administration No reactions were encountered with the use of this solution

The chemical methods for the determination of glucose and pyruvate have been previously described [6] Lactic acid was determined by the method of Barker and Summerson [7]

RESULTS

**Pyruvate** There was no significant change in the fasting pyruvic acid level during 30 min of anesthesia prior to the start of the pyruvate infusion (Fig 1) The average rise in pyruvic acid during the first 30 min of pyruvate infusion was 1 33 mg /100 ml for the control and 3 13 mg /100 ml for the anesthesia tests At the end of 60 min infusion the mean rise above fasting was 3 32 mg /100 ml for the control tests and 2 61 mg /100 ml for the anesthesia tests When the infusion was stopped there was a prompt return to the fasting level in the control tests whereas in the anesthesia study the pyruvic acid was still 0 55 mg /100 ml above the fasting level 2 hr after the infusion and ether were discontinued The differences between the tests at *60 150 180 and 210 min have all proved to be significant by application* of the Fisher t test

**Lactate** When pyruvate is given there is a rise in blood lactic acid The changes in lactic acid during the administration of pyruvate with and with

PYRUVATE TOLERANCE BEFORE AND DURING ETHER ANESTHESIA

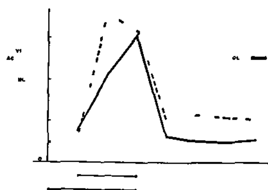


Fig 1

*Nutritional Biochemical Company of Cleveland
†Courtesy of Meade Johnson Company

# THE EFFECT OF ANESTHESIA ON BLOOD VOLUME AND OSMOTIC PRESSURE *

WILLIAM R. DRUCKER, R. SHULTS, C. COSTLEY, J. DOCKERY
AND WILLIAM D. HOLDEN

Homeostasis in living organisms depends to a large extent on the maintenance of physiological osmotic pressures. The distribution of water and permeable solutes throughout plants and animals depends on osmotic effects. Many of the disorders in body fluid metabolism are explainable on the basis of changes in osmotic pressure.

In 1955 Moore and his associates reported that the osmotic pressure of plasma increased during the course of ether anesthesia. It is known that ether causes many physiologic disturbances. Some of these, such as decreased renal excretion of water, may be a response to an increase in the osmotic pressure of plasma. It seemed desirable, therefore, to determine the basis for the rise in osmotic pressure during ether anesthesia and whether it could be correlated with any measurable physiologic change.

## METHOD

Ten patients hospitalized for rehabilitation at Highland View Cuyahoga County Hospital volunteered to undergo 90 min. of ether anesthesia without surgery. All patients received demerol 50 mg. and atropine 0.64 mg. as preanesthetic medication. Samples of venous blood were drawn through a Cournand needle placed in a forearm vein. The first sample was drawn prior to the start of anesthesia. Subsequent samples were obtained every 30 min. during anesthesia and for 2 hr. after discontinuation of anesthesia.

The anesthesia was induced by intravenous administration of 150 to 300 mg. of thiopental sodium. Ether anesthesia was started with a mixture of nitrous oxide, oxygen and ether and maintained with endotracheal oxygen-ether. All anesthetics were administered by the same person (C.C.). During the final 60 min. of ether each subject received an intravenous infusion of sodium pyruvate or sodium lactate by means of a constant rate infusion pump as part of another study. During anesthesia capillary oxygenation was checked by an ear oximeter. Blood and plasma volume, hematocrit, plasma sodium concentration and blood oxygen saturation were determined prior to the start of anesthesia and 30 and 90 min. after surgical anesthesia was reached. An electrocardiogram was obtained the day before each study and frequently during the course of anesthesia. Blood pressures were obtained by a sphygmomanometer. Urine was collected from 7 patients every half hour by an indwelling catheter and the urine was measured for volume and sodium concentration.

The blood for osmolarity determinations was placed in refrigerated tubes with dried heparin. The tubes were centrifuged and the plasma pipetted into a small tube which was corked and stored at −11°C. This was done to

*From the Departments of Surgery, Western Reserve University School of Medicine, Highland View Hospital and University Hospitals of Cleveland, Cleveland, Ohio. Work supported in part by U.S.P.H.S. grant number A 1253.

With the technical assistance of Nancy Hofmann and Helen Bassett, and the assistance of Warren Strauss with the illustrations.

mine 2, lipoic acid 3 magnesium or manganese and 4 DPN (diphospho pyridine nucleotide) (Fig 3) It is unlikely that thiamine deficiency was responsible for the disturbed pyruvate metabolism since these patients received nutritionally adequate diets with large supplementary doses of vitamin B complex and had normal blood thiamine levels with no clinical evidence of avitaminosis There is insufficient evidence to allow speculation on the relationship of lipoic acid and magnesium or manganese to anesthesia

The fourth factor in the oxidative decarboxylation of pyruvic acid to acetyl CoA is oxidized DPN Should there be some interference in the cytochrome system which would inhibit the reformation of DPN-ox from DPH H increased quantities of pyruvate might be reduced to lactate The reduction of pyruvate to lactate would form DPN ox The renewed supply of DPN ox would allow conversion of more pyruvate to acetyl CoA The increased rise in lactate found in this study is compatible with this concept

The cause of the interference with the reformation of DPN-ox remains to be determined Anoxia *per se* is an unlikely cause since all of the patients had normal blood oxygen saturation during anesthesia Also studies on tissue slices have shown that narcotics in therapeutic doses do not interfere with the uptake and activation of oxygen by living cells [8] Quastel[5] has postulated that narcotics produce a block between DPN and cytochrome

In summary, it cannot be stated on the basis of this study where the block occurs in pyruvate metabolism during ether anesthesia Possible mechanisms have been discussed It is known that cells depend on the combustion of pyruvate in the citric acid cycle for most of their energy It is tempting to speculate therefore that the state of ether narcosis in humans may be due to decreased cell energy as a result of impaired utilization of pyruvate

## CONCLUSIONS

1 Tolerance for exogenous sodium pyruvate is diminished in humans during ether anesthesia

2 The site of the block in pyruvate metabolism in anesthetized humans must await further study for elucidation

3 The impaired metabolism of pyruvate during ether anesthesia may be sufficient to diminish the energy production necessary for functional activity of neural cells This may be sufficient to induce the state of anesthesia

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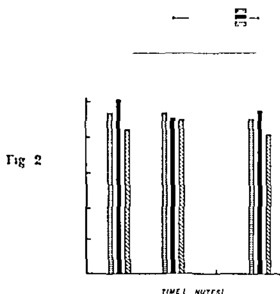



Fig 2

motic pressure The urinary sodium concentration decreased after the in duction of ether anesthesia and remained considerably reduced thereafter

All patients were awake and able to talk before the termination of the study There was no change in capillary oxygen saturation by ear oximetric readings Blood analysis revealed normal oxygen saturation for all cases during anesthesia

Plasma with an elevated osmotic pressure showed a considerable fall in the osmotic pressure when this plasma was allowed to stand at room tem perature or shaken or heated slightly There was no change in the osmotic pressure of the control plasmas with similar treatment

DISCUSSION

Effective osmotic pressure is that fraction of the total osmotic pressure which governs movements and distribution of water between cells and extra cellular fluid Sodium contributes the largest component of effective plasma osmotic pressure because of its high concentration in extracellular fluid and its relative exclusion from cells During anesthesia without associated sur

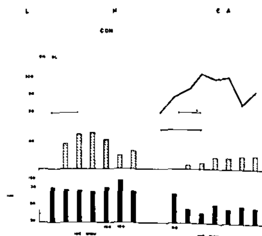


Fig 3

PLASMA OSMOTIC PRESSURE HEMATOCRIT AND BLOOD VOLUME DURING ETHER ANESTHESIA

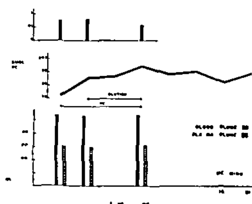


Fig 1

prevent the volatilization of ether from the plasma The osmolarity determinations were subsequently performed by the freezing point depression method with the Fiske osmometer

Determinations of blood and plasma volume were done by the method described by Storaasli *et al* [1] using $I^{131}$ tagged human albumin Arterial hematocrits were determined by the method of Wintrobe Plasma and urine sodium concentrations were obtained by the use of a Beckman flame photometer and oxygen was determined by a Van Slyke manometric gas analysis apparatus

RESULTS

The average values of the venous osmolarities determined every 30 min during the course of this study are illustrated in Figure 1 There was a rise of 13 mOsm during the first 30 min and an additional mean rise of 9 mOsm during the final 60 min of ether anesthesia Measurements made during the 2 hr after ether was discontinued demonstrated a fluctuating but persistently elevated plasma osmotic pressure

In contrast to the 8 per cent rise in plasma osmolarity with ether there were no significant changes in blood volume plasma volume hematocrit or plasma sodium In order to rule out the possibility that administration of sodium containing fluids (98 mEq of Na) prevents a fall in plasma sodium 5 additional patients were studied who received a 10 per cent solution of sugar in water The average sodium concentration for these patients is compared with the average sodium concentration for the patients who received sodium lactate or sodium pyruvate (Fig 2) There is no significant difference in the 3 groups The patients who received sugar solutions showed a rise in osmolarity comparable to the rise found with lactate and pyruvate infusions with ether anesthesia but no change in blood or plasma volume or hematocrit

Control tests were run a day or two prior to anesthesia for 7 patients in which they were given an amount of fluid similar in volume and sodium concentration to that given during anesthesia The marked depressant effect of ether on salt and water excretion is illustrated in Figure 3 An interesting observation is that although the urine half hourly volume abruptly decreased during anesthesia it began to rise again despite a rising plasma os

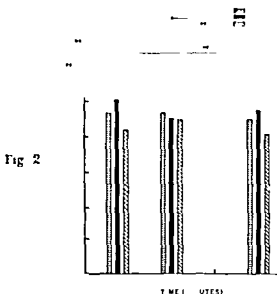



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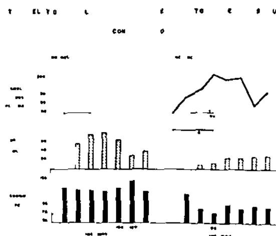


Fig 3

PLASMA OSMOTIC PRESSURE HEMATOCRIT AND BLOOD VOLUME DURING ETHER ANESTHESIA

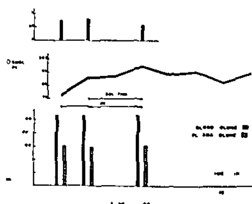


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# RESPIRATORY ACIDOSIS AND PULMONARY VENTILATION DURING OPEN THORACOTOMY

## *The Effect of Compression of the Lung*

THOMAS F. NEALON, JR., JOYCE F. PRICE AND JOHN H. GIBBON, JR.

The respiratory acidosis which occurs during operations is due to inadequate pulmonary ventilation. Such acidosis can be avoided by assisting the respiratory efforts of the patient or, preferably, by ventilating the lungs mechanically with no respiratory effort on the part of the patient. When the chest is opened it has been shown that the pulmonary ventilation must be considerably increased to avoid respiratory acidosis.[1] The following study was undertaken to determine why an increase in pulmonary ventilation was necessary. With an open thoracotomy the increase in total pulmonary ventilation necessary to prevent respiratory acidosis was found to be the result of compression of the lung.

### METHOD

Six patients were studied during operations requiring an open thorax (Table I). Anesthesia was induced with intravenous thiopental sodium and succinylcholine. After introducing an endotracheal tube, anesthesia was continued with ether or nitrous oxide and oxygen. The patients were made apneic and pulmonary ventilation was carried on by an intermittent positive and negative pressure ventilator (The Jefferson Ventilator). Apena was produced by hyperventilation in 5 patients and by succinylcholine in Case #4.

The sampling apparatus and techniques used have been described in detail in a previous publication.[2] In brief, a mixing chamber and a gas flow meter were incorporated in the expiratory line of the closed rebreathing anesthetic circuit. Thus mixed expired gas samples could be obtained and the ventilation measured. An infra-red absorption analyzer gave a continuous indication of the carbon dioxide in the respired gases in the endotracheal tube.[3] Using the carbon dioxide analyzer as a monitor, the ventilation was adjusted until the carbon dioxide tension ($pCO_2$) of the expired gases lay at some point between 35 and 40 mm. Hg with 1 exception (Case #4). The ventilation was adjusted by changing the tidal volume. This was accomplished by increasing or decreasing the difference between the positive and negative pressures on the ventilator. A "steady state" was considered to exist when the $pCO_2$ of the end expired gas had remained at a constant value for 10 min. with no change in pulmonary ventilation. The pulmonary ventilation was then measured while a sample of arterial blood was drawn. The $pCO_2$ and $O_2$ saturation of the arterial blood were determined. These measurements were made: (1) after the operation was under way but before the chest was opened; (2) after the chest was opened but before the lung was disturbed; (3) during the intrathoracic portion of the operation which necessitated some compression of the lung, and (4) in 3 patients after the lung was re-expanded.

### RESULTS

The results are tabulated in Table I and illustrated graphically in Case #2 by Figure 1. In all patients the ventilation required to maintain the

gery there is no significant loss of sodium from the body either externally or into a third space The plasma sodium concentration should therefore remain unaltered providing there is no loss or gain of fluid into the extracellular space But if some agent were added to the plasma which increases the effective osmotic pressure the extracellular fluid volume should rise due to a transfer of water from the cells This in turn should be reflected by a fall in the sodium concentration The fact that neither a measurable rise in blood or plasma volume nor a fall in sodium concentration or hematocrit was found in this study despite a rise of 22 mOsm in plasma osmotic pressure suggests that the increase was not due to a substance which remained confined to the extracellular space

The renal response to an increase in effective plasma osmolarity is a reduction of urinary volume If dehydration is also present there is a decreased excretion of sodium as part of the dehydration reaction [2] The observed rise in osmolarity during ether anesthesia theoretically could have a causal relationship to the decreased renal excretion of water and sodium Although a marked reduction in renal excretion of water and sodium was found during anesthesia there was an increase in half hourly urine volumes despite a rising osmotic pressure This observation also supports the hypothesis that the rise in osmotic pressure was due to a substance distributed across both extra and intracellular compartments

The fact that the elevated osmotic pressure falls when the plasma either stands at room temperature or is heated slightly is evidence that ether a highly volatile substance is responsible for the increase in osmotic pressure

## SUMMARY AND CONCLUSIONS

1 The increase in plasma osmotic pressure with ether anesthesia is probably due to ether incorporated in the plasma

2 The increase in plasma osmotic pressure is not associated with a measurable alteration of blood or plasma volume hematocrit or sodium concentration Therefore the diminished renal excretion of water and sodium during ether anesthesia cannot be explained by the elevated osmolarity of the plasma

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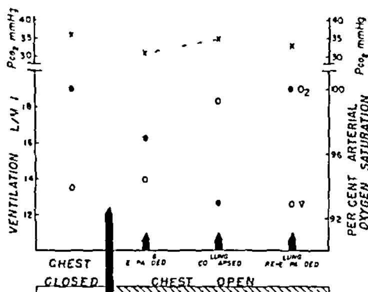



Fig 1 Graphs of the $pCO_2$ and per cent saturation with oxygen of arterial blood and the pulmonary ventilation in Case No 2 The $pCO_2$ was maintained at approximately the same level throughout The pulmonary ventilation with the chest open and the lung collapsed had to be markedly increased to avoid a rise in the $pCO_2$ With the chest open and the lung expanded the pulmonary ventilation could be kept at approximately the same level as when the chest was closed without any rise occurring in the $pCO_2$. The oxygen saturation fell slightly when the chest was opened and the lung expanded but was still within the normal range With the lung collapsed despite the increase in ventilation there was a significant decrease in the saturation which rose again to normal levels when the lung was re expanded with the chest still open

$pCO_2$ of the arterial blood at the same level was approximately the same before and after the chest was opened Compression of the lung however necessitated a considerable increase in ventilation to keep the $pCO_2$ at the same level with one exception (Case #6) With the lung compressed even with hyperventilation there was in most cases a significant drop in the $O_2$ saturation of arterial blood In the 3 instances in which the measurements were repeated after the lung was re-expanded it was again possible to maintain the $pCO_2$ at the same level with the former and lesser pulmonary ventilation

DISCUSSION

In these observations an open thoracotomy did not in itself result in the need for increased pulmonary ventilation to avoid a rise in the $pCO_2$ of arterial blood Only after the lung was compressed was it necessary to increase the ventilation to prevent the $pCO_2$ from rising The smallest increase was required in Case 6 the repair of a hiatal hernia where only slight compression of the lower lobe of the left lung was used

During compression of the lung some blood will pass through capillaries in the walls of collapsed alveoli This in effect results in an anatomic shunt of some unaltered venous blood through the pulmonary circulation to the

# Table 1

| CASE | OPERATION | pCO ARTERIAL BLOOD MM HG | | | | VENTILATION L/MIN | | | | ARTERIAL OXYGEN SATURATION PER CENT | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CHEST CLOSED | CHEST OPEN | LUNG COMPRESSED | LUNG REEXPANDED | CHEST CLOSED | CHEST OPEN | LUNG COMPRESSED | LUNG REEXPANDED | CHEST CLOSED | CHEST OPEN | LUNG COMPRESSED | LUNG REEXPANDED |
| 1 | Lobectomy | 35 | 32 | 32 | 33 | 16.0 | 15.6 | 22.0 | 15.4 | 92 | 91 | 91 | 90 |
| 2 | Excision Neurofibroma | 36 | 31 | 35 | 33 | 13.5 | 14.1 | 18.3 | 12.6 | 100 | 97 | 93 | 100 |
| 3 | Excision Aortic Aneurysm | 35 | 38 | 35 | | 20.0 | 19.0 | 23.9 | | 100 | 98 | 95 | |
| 4 | Lobectomy | 58 | 59 | 65 | 53 | 16.2 | 15.8 | 20.4 | 12.9 | 96 | 96 | 87 | 97 |
| 5 | Excision Aortic Aneurysm | 39 | 38 | 41 | | 17.1 | 16.8 | 17.5 | | 97 | 98 | 93 | |
| 6 | Repair Hiatal Hernia | 36 | 34 | 36 | | 9.9 | 11.6 | 11.9 | | 98 | 98 | 92 | |

# FAILURE OF THE CIRCULATION IN ACUTE HYPOXIA*

IBRAHIM K. DAGHER AND GEORGE H. A. CLOWES JR

Experimentally, hypoxia resulted in standstill of the heart when the arterial oxygen content fell below three volumes per cent[1] On the other hand cardiac arrest from pure hypercapnia did not occur in intact animals[1,7]

The present study was conducted in dogs subjected to acute anoxia to determine which portion of the circulation fails first It is the purpose of this paper to present evidence that the heart failed under these conditions while the peripheral resistance and venomotor tone remained normal or increased

## METHOD

The aortic blood flow (exclusive of coronary circulation) was measured with a Shipley rotameter[2] that recorded on a Grass Ink writing Oscillograph Model III B[6] through a demodulator The blood was directed to the rotameter by way of an Eckstein cannula[3] Simultaneous electroencephalographic and electrocardiographic tracings (lead II) were made on separate channels of the same oscillograph Left auricular right auricular and pulmonary artery pressures were measured with water manometers while femoral artery pressure was recorded by a mercury manometer

The carbon dioxide and oxygen contents of arterial blood samples were determined by the method of Neill and Van Slyke[9]

The left pleural cavity of 7 mongrel dogs anesthetized with sodium pentobarbital was entered by excising the third and fourth ribs The thoracic aorta was mobilized from the arch down to the level of the fifth rib and the 2 innominate vessels were freed The left carotid artery was isolated through a separate incision made in the neck Coagulation of blood was prevented by the intravenous administration of 5 mg of heparin per kg of body weight The left carotid artery was cannulated and ligated distally The cannula led to a reservoir placed at a height of 2 m above the level of the heart The left pulmonary artery and the left auricle were cannulated through segmental pulmonary vessels The left femoral artery was cannulated and attached to a mercury manometer The venous pressure was measured through a catheter placed in the inferior vena cava

The freed portion of the aorta was severed between Potts clamps and an Eckstein cannula was placed between the 2 aortic segments During the installation of the aortic cannula in order to prevent cerebral hypertension blood was allowed to run into the reservoir attached to the left carotid artery and as soon as the Potts clamps were released the blood was pumped back into the animal The Eckstein cannula directed the blood through the Shipley rotameter and back to the aorta A side branch of that cannula was introduced into the right innominate artery to maintain the blood circulation to the brain as both innominate vessels were ligated thus shunting all the aortic blood flow (exclusive of coronary circulation) to the rotameter (Figure 1)

*From the Department of Surgery Western Reserve University School of Medicine at City Hospital of Cleveland Ohio This work supported in part by Grant Number H 1317 (c3) from the National Institute of Health

left side of the heart Under these circumstances, if the pulmonary ventilation is constant the $pCO_2$ of arterial blood will rise and the oxygen saturation will fall This rise and fall will be roughly proportional to the amount of lung collapsed Hyperventilation prevents the rise in $pCO_2$ of arterial blood but does not prevent a fall in oxygen saturation The hyperventilation washes out a more than normal amount of $CO_2$ in the blood passing through functioning alveoli thus compensating for the unaltered amount of $CO_2$ in the shunted venous blood The hyperventilation however does not result in a significant increase in the amount of oxygen held by the blood passing through the functioning alveoli The hemoglobin in this blood is already completely saturated, or almost so and the amount of oxygen in physical solution (normally 0 15 ml /100 ml of blood) will not be significantly increased by the hyperventilation From a clinical standpoint the surgeon should be aware that while part or all of the lung is collapsed hypercapnia can be avoided by hyperventilation but that hyperventilation will not relieve the associated hypoxemia Periodic re expansion of the lung is probably advisable to provide intermittent normal saturation of the arterial blood with oxygen

## SUMMARY

1 In 6 patients the total pulmonary ventilation, carbon dioxide tension and per cent saturation of arterial blood with oxygen were determined during steady states in the course of operations involving an open thoracotomy

2 No increase in pulmonary ventilation was required to maintain the carbon dioxide tension of arterial blood at a constant level with the chest open and the lung expanded

3 Compression of the lung with one exception required an increase in pulmonary ventilation to maintain the carbon dioxide tension of arterial blood at the same level

4 With the lung compressed even with hyperventilation in most cases the per cent saturation of arterial blood with oxygen fell

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on the mean systemic arterial blood pressure consisted of a progressive rise to about 125 per cent of the control reached in about 1 min after the induction of hypoxia This rise was momentary and was followed by a gradual and progressive drop that reached the zero level in about 6 min

The pressure in the left auricle and in the pulmonary artery progressively rose up to a peak that was reached in about 2 min This was followed about 1 min later by a progressive rise in the vena cava pressure This rise maintained itself at the same level till the end of the experiment

The heart rate steadily fell from the start of the experiment and reached its lowest level in about 3 min At this point the heart became dilated and it came to a standstill in about 6 min despite the presence of a recordable electrocardiographic activity several minutes thereafter

As illustrated in graph 2 Figure 3 the aortic blood flow steadily and progressively decreased until it stopped in about 5 minutes

In contradistinction to the decrease in the blood flow the total systemic arterial peripheral resistance calculated after the formula of Fowler *et al*[4] increased markedly until circulation of blood ceased

*DISCUSSION*

The progressive decrease in the systemic arterial blood pressure after the initial rise associated with a concomitant decrease in the blood flow speaks in favor of the inefficiency of the left ventricle in maintaining its role as a mechanical pump The increase in the venous pressure is indicative of right ventricular failure

Study of the time relationship between the failure of the left and right ventricles reveals that the pressure in the left auricle rises about 1 min after the systemic arterial blood pressure starts to drop the rise in the pulmonary artery pressure comes about 20 sec after the rise in left auricular pressure The venous pressure increases about 1 min after the rise in the pulmonary

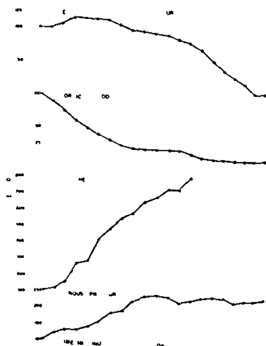


Fig 3 Time relationship between the mean arterial blood pressure aortic blood flow peripheral resistance and venous pressure expressed in percentage of the control values obtained before the induction of hypoxia

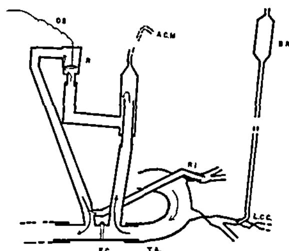



Fig 1 Illustration of Experimental Set up OS To Oscillograph R Rotameter A C M To Air Chamber Dumper B R Blood Reservoir R I Right Innominate Artery L C C Left Common Carotid E C Eckstein Cannula T.A Thoracic Aorta

Control readings were made after equilibrium had been established for a period of 10 min The animal was then made hypoxic by the administration of a respiratory mixture containing 99 5 per cent nitrogen and 0 5 per cent oxygen through the intratracheal tube Pulse electroencephalogram electro cardiogram aortic blood flow and pressures in left auricle inferior vena cava and femoral and pulmonary arteries were continuously recorded Arterial blood samples were drawn at intervals of 2 to 3 min for analysis

## RESULTS

Under the experimental conditions the arterial blood oxygen content pro gressively fell to 0 5 volume per cent within 5 min after the start of nitrogen administration The arterial $pCO_2$ remained near normal until circulation ceased

As shown in graph 1 Figure 2 the effect of this acute and severe hypoxia

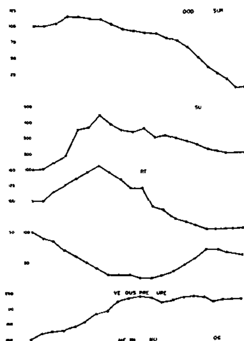


Fig 2 Time relationship between the mean arterial blood pressure pressure in left auricle and pulmonary artery heart rate and venous pressure expressed in percentage of the con trol values obtained before the induction of hypoxia

# DYNAMICS OF PLEURAL EFFUSIONS*

ROY H. CLAUSS, HACOP YACOUBIAN AND HAROLD G. BARKER

The hydrodynamic factors governing the accumulation, maintenance and resolution of pleural effusions are largely unknown. The underlying diseases which lead to such collections and the usual clinical course are better known but the basic physiology may seem dissimilar in varying clinical conditions. It is apparent that if differing hydrodynamic patterns could be found to characterize the various states, an approach would be made to a better understanding of the physiology. With this in mind we have studied 20 patients who had pleural effusions secondary to tuberculosis, carcinoma, nontuberculous infection, and spontaneous pneumothorax. To date some groups are too small to permit conclusions but as yet no characteristics have emerged which distinguish the hydrodynamics of the various groups.

## METHOD

Deuterium oxide was used as a tracer for water in the closed 2 compartment system, where 1 compartment is the pleural space and the other is the remainder of the total body water. In some cases the tracer was injected intrapleurally and in others it was injected intravenously. In either case a figure for the volume of the sum of the two compartments (total body water) is easily calculated so long as equilibrium is reached. Where the tracer is injected intrapleurally, a figure for the volume of the primary compartment (pleural fluid) can easily be calculated by extrapolation of the semilog plot of the concentrations of the tracer in pleural fluid samples. Where the primary compartment is all the body water except the pleural fluid (intravenous injection) the calculation of the volume of the primary compartment by extrapolation becomes inaccurate due to the slow rate of change in concentrations. We found it preferable in these cases, therefore, to determine the pleural fluid volume instead using an independent tracer (T 1824 dye). Likewise, it was deemed advisable to base the measurement of half life on the slope of the semilog plot of concentrations of the tracer in pleural fluid whether the pleural fluid represented the primary compartment (compartment of injection) or the secondary compartment (compartment of diffusion).

The early experiments demonstrated that equilibrium between the compartments is usually achieved within 12 hr, occasionally earlier but sometimes not until about 24 hr. Patients who had normal cardiodynamics were selected and they were studied under conditions of normal hydration. Using local anesthesia, polyvinyl tubing was introduced into the pleural space through a thin walled 14 gauge needle and the needle then withdrawn. A needle adapter and three way stopcock were attached and 20 mg of heparin injected to prevent coagulation. After drawing a control sample of small volume a volumetrically measured quantity of Evans blue dye was injected intrapleurally followed by an accurately weighed dose of heavy water (ap

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artery pressure In other words, the left ventricle fails first and, subsequently back pressure causes the right ventricle to fail

The initial rise in the systemic blood pressure obtained in this experiment is comparable to the results of Gellhorn[5] who used 8 5 per cent oxygen and nitrogen mixture in the human and to those of Clowes *et al*[1] who used 0 5 to 1 per cent oxygen and nitrogen mixture in the dog

The left ventricular failure starts by bradycardia as illustrated in graph 2 Subsequently the bradycardia leads to a mechanical standstill of the heart This occurs despite recordable electrocardiographic activity These results support the teaching that the common myocardium (the contractile portion of the heart) will not function in the absence of oxygen while the specific tissue (sinus node auriculo ventricular node and bundle of His) will function rhythmically without oxygen[8]

The total systemic arterial resistance rose throughout the experiment as long as blood flow continued This means that in acute and severe hypoxia the peripheral vasoconstrictor mechanisms do not fail but display over activity as evidenced by the increase of the peripheral resistance and the forwarding of adequate venous return leading to an increased venous pressure

## SUMMARY AND CONCLUSION

From the data obtained from dogs subjected to acute severe hypoxia it is concluded that the primary cause of circulatory arrest is failure of the heart to perform its work rather than a fall in arterial resistance or venous tone The mechanical failure of the heart occurs despite recordable electrocardiographic activity

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formulae used above have been derived by others (in slightly different form) who have written on the closed two compartment system [1,2,3]

### RESULTS AND DISCUSSION

The results are presented in Table 1 We encountered occasional patients in whom equilibrium was prolonged beyond 24 hr or was never achieved at all Such patients were found to retain $D_2O$ and/or Evans blue in their pleural space at unreasonably high concentrations and it could only be assumed that their effusions were loculated thus preventing the collection of representative samples These subjects have been eliminated from the report. In the 2 subjects with spontaneous pneumothorax an artificial pleural effusion was created by the injection of isotonic saline solution to see if the turnover rate might be different from that in patients with spontaneous effusions It is seen that the rate is of the same general order of magnitude It should be noted that there is considerable individual variation in the exchange rates and this does not seem to depend upon the size of the effusion or upon the disease process involved although too few studies have been done in non tuberculous effusions to be sure about this

The high rates of turnover found (30 to 75 per cent per hour) are of interest since they are similar to the high rates of turnover described for ascitic fluid [2] In a few patients we have also studied the turnover rate of sodium in pleural fluid using $Na^{24}$ as a tracer and have found that the turnover rate is somewhat slower than that of water Total body water in some of our subjects was found to be higher percentagewise than one might expect but this was felt to be due to loss of body fat with their disease rather than additional body water accumulation over and above the pleural fluid

### SUMMARY

Rate of pleural fluid turnover has been measured by a tracer technique in a group of patients with pleural effusions from several different disease processes We have not been able to detect a difference in exchange rate charac

*Table 1 Pleural Fluid Exchange Rates*

| | | | PLEURAL FLUID | | |
|---|---|---|---|---|---|
| | | | | EXCHANGE RATE | |
| PT | AGE | SEX | VOL (L) | L/HR. | %/HR |
| W.S | 61 | M | 1.0 | .30 | 30 |
| C K | 45 | M | 1.2 | 49 | 41 |
| R C. | 29 | M | 2.4 | 72 | 30 |
| W L | 58 | M | 1.8 | 1.04 | 58 |
| M V | 24 | F | .6 | 19 | 32 |
| J C | 33 | M | 2.4 | 72 | 30 |
| W M | 21 | M | 1.4 | 61 | 44 |
| M F | 20 | F | 1.5 | 74 | 49 |
| F J | 39 | F | 2.7 | 1.45 | 54 |
| J L | 32 | M | 1.3 | 69 | 53 |
| S M | 37 | M | 1.8 | 71 | 39 |
| F T | 29 | M | 1.1 | 83 | 75 |
| H B | 37 | M | 1.2 | .68 | 57 |

E J had carcinoma J L Friedlander type B pneumonia S M constrictive pericarditis E T *and* H B artificial effusions the remainder all had tuberculous effusions

proximately 50 gm ) either intrapleurally or intravenously Mixing of the contents of the pleural effusion was then accomplished by a standard exer cise of two deep breaths in each lateral position upright and prone The patients then rested supine A multiple syringe technique was employed for removal of specimens and the volumes of pleural fluid removed were as small as possible (no more than 3 or 4 cc each) Specimens were withdrawn at 20 to 30 min intervals for 4 samples and then at longer intervals up to 24 hr Simultaneous pleural fluid and blood samples were drawn throughout in some subjects and in others bloods were drawn only near the conclusion to demonstrate equilibrium A pre test overnight fast was continued until after the first 4 specimens had been obtained after which fluids and foods were given at a rate approximating loss No fluids were permitted in the 2 hr preceding any sampling Evans blue determinations were carried out in a Beckman model DU spectrophotometer Heavy water was determined by the standard falling drop method following distillation of pleural fluid and serum samples Calculation of pleural fluid volume from Evans blue dye was based on straight dilution since its concentration did not decline appre ciably with time but several samples were always drawn and analyses aver aged Total body water was calculated from the $D_2O$ figures by the usual method converting weight of dose given into volume at body temperature using the figure for percent purity of the standard and known data for spe cific gravity of heavy water at body temperature A very small correction was made for assumed urinary and insensible loss of dose in the calculation of total body water since this calculation was based on the 12 or 24 hr equi librium samples No similar correction was made however in the earlier figures which went into the determination of the half life slope and intercept on the semilog plot

For the calculation of exchange rate of pleural fluid the individual con centrations of tracer in pleural fluid were subtracted from the equilibrium figure *(in the case of intravenous administration) and the resulting* data plotted on semilog paper against time When $D_2O$ was injected intrapleu rally the subtraction was reversed The slopes and intercept of the resulting lines were then read in the latter case and only the slope in the former Calculations were based on the following formulae

$$(1)\ P_t = (P_o - P_{q})e^{-(K_p + K\ )t} + P_{q}$$

$$(2)\ S_t = -S_{q}e^{-(K_p + K\ )t} + S_{eq}$$

$$(3)\ P_t - S_t = P_o e^{-(K_p + K\ )t}$$

where P is concentration in primary compartment

S secondary

*t o and eq are times*

$K_p$ is fraction of the volume of primary compartment exchanged per unit time

K is the same for the secondary compartment

It is obvious from Equation 2 that the intercept of the straight line will be at the equilibrium concentration thus making it impossible to calculate the volume of the secondary compartment based on data in this compartment alone and necessitating the use of the independent tracer Evans blue The

METHOD

The veins used were taken from dogs under strictly sterile techniques Each vein was placed in a glass tube previously sterilized by autoclave, which contained 2 cc of Ringer s solution 500 000 u of penicillin and ½ gm of dihydro-streptomycin The tubes containing the veins were immediately placed in the freezer where they were kept at 8 to 10°C below zero From the time that the vein was extracted for preservation small fragments were periodically subjected to careful histologic study and bacteriological control Before being taken to the operating room for use the fragments of vein were subjected to a final histologic and bacteriological study (Fig 1)

During the course of this experiment 200 dogs varying in weight from 8 to 25 kg and in age from 1 to 7 yr were anesthetised Ether or nembutal was used as anesthesia the latter dosage being from 1 to 6 cc /kg of weight given intraperitoneally and using in both cases intratracheal oxygen positive pressure with our own design semi automatic anesthetic machine for intra thoracic work in animals

The left hemithorax was then opened by sub periosteal resection of the 5th rib A left pneumonectomy was then performed using the usual tech nique In 100 dogs the left bronchus was closed by separated stitches of #00 Pagenstecher linen The fragments of superior vena cava had been care fully thawed first by Balneum Marine and then by the application of warm compresses The open fragment was then placed over the bronchial stump in the form of a cap covering it well and then sutured to the external bronchial wall by separate stitches of Deknatel #5 0 We have also used continuous stitching with the same material and obtained very satisfactory results

After we made sure that the vena cava completely covered the bronchial stump and that it was correctly sutured positive pressure was augmented considerably to assure that the technique had been correctly applied and that there was no escape of air even under exaggeratedly high pressure

The thoracic wall was closed in the usual manner The dogs received postoperative penicillin intramuscularly 100 000 u each 12 hr during the first 6 days

The other 100 dogs were used as a control group

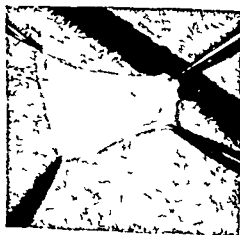


Fig 1 Vena cava graft opened and extended ready for use

terizing different diseases Likewise the rate in 2 patients with normal pleurae was measured after creation of artificial effusions by the injection of saline solution and found to be similar to the rate in patients with spontaneous effusions

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# THE USE OF FROZEN VENA CAVA GRAFTS TO AID THE CLOSURE OF THE BRONCHIAL STUMP FOLLOWING PNEUMONECTOMY*

ALFONSO TOPETE ROBERTO VAZQUEZ PALLARES J TRINIDAD PULIDO IGNACIO OCHOA MARTINEZ SALVADOR RODRIGUEZ ANDRÉ ESTEVES LIMA AND SALVADOR DIAZ GUTIERREZ

One of the problems that still vexes resectional pulmonary surgery is the appearance of bronchial fistulas especially as the aftermath of extirpation of the superior lobes and pneumonectomies in tuberculous patients Diverse methods of covering the sutured bronchi and even special techniques for suturing have been reported

As has been observed by various authors in a large number of patients the percentage of success is high whether the method used be that of recovering the bronchi with peri bronchial tissue or with parietal pleura pericardium fatty tissue et cetera We must accept the fact however, that a number of patients develop total or partial ruptures of the bronchi after resection

The object of this paper is to report the following experimental study in which dogs subjected to pneumonectomy were treated by covering the stump of the sutured bronchi with pieces of the superior vena cava which has been preserved by freezing This study covers a period of 3 years and was done at the Department of Surgical Investigation of the University of Guadalajara Mexico

The reasons that persuaded us to decide upon the use of the vena cava to cover the sutured bronchi were the histologic manifestations of fibrous reaction and organization observed in arterial and vein grafts used as vascular substitutions, and consistence of the venous tissue

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ing two dogs presented fistulas and were sacrificed for observation In both purulent liquid was found in the cavity When sacrificed the general condition of these dogs was very poor

**Examination of the Stump** In a gross study, the group where the stump was capped by vena cava grafts showed considerable firmness in the area of the stump after the third day This aspect was progressive during the following days, and by the end of the tenth day had acquired a very consistent aspect The control group also tended to rapid firmness, but we believe that there was greater consistence in the first group (Figs 2a, 2b, 2c)

**Microscopic Study** Usually after 24 hr at the point of contact of the vena cava with the bronchi there could be observed a fibrous exudation which englobed inflammatory cells after 48 hr, inflammatory tissue with young fibroblasts and great capillary organization was observed on the external surface of the vein As time passed the conjunctive proliferation became more intense and firm the fibrinous exudation disappearing until there remained a true fibrous frame (Fig 3a 3b 3c 3d)

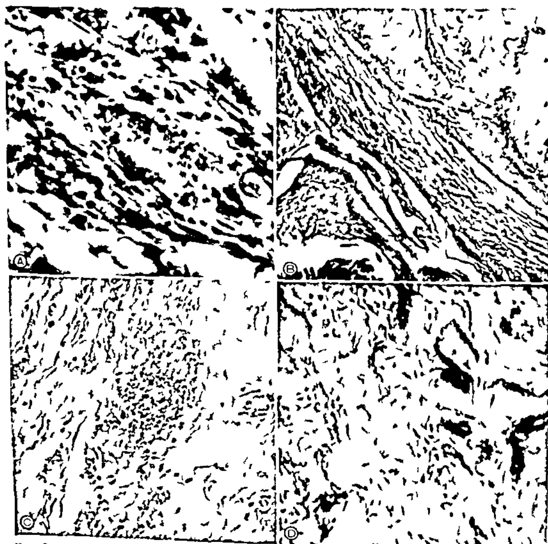



Fig 3 (a) Young fibroblastic exudation with inflammatory cells and capillary organization 48 days after bronchial venous covering 3 (b) Fibrous organization after 10 days 3 (c) Healing process at 60th day 3 (d) Conjunctive sclerosis at 105 days

*Table 1 Experiments Bronchial Closure in Pneumonectomy*

| NO OF DOGS | DEATHS | FISTULAE |
|---|---|---|
| 1—WITH AID OF VENA CAVA GRAFT | | |
| 100 | 8 8% | 0 0% |
| 2—WITHOUT VENA CAVA GRAFT | | |
| 100 | 12 12% | 5 5% |
| Total 200 | 20 20% | 5 5% |

They were also subjected to left pneumonectomy, the same surgical technique being used as with the other group However in this control group the bronchial stump was not capped with frozen vena cava grafts but was covered by peribronchial tissue

Both groups were subjected to the same postoperative treatment All received postoperative penicilin for 6 days Over post surgical periods varying from 24 hr to 8 mo a number of animals was sacrificed for study

DISCUSSION

**Mortality** In the group subject to pneumonectomy in which the bronchial stump was capped by frozen vena cava grafts there were 8 deaths due to pneumonitis atelectasis and cardiac failure (8 per cent) In the control group there were 12 deaths 7 from pneumonitis and atelectasis and 5 from empyema with bronchial fistula (12 per cent)

**Morbidity** In the group where the bronchial stump was capped by frozen vena cava grafts no case of bronchial fistula appeared and the formation of liquid in the cavity was noticeably reduced

In the control group where the bronchial stump was covered with peribronchial tissue 5 bronchial fistulas occurred 3 of which provoked empyema which we interpreted as leading to the death of these animals The remain

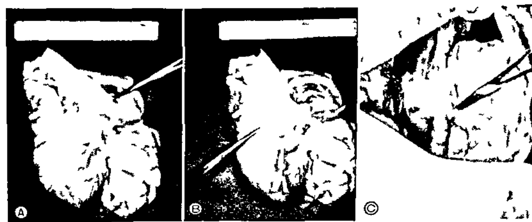



Fig 2 (a) Dog sacrificed 105 days after left pneumonectomy showing venous covering of the left main bronchus 2 (b) Specimen with trachea and main bronchus opened longitudinally 2 (c) Left pleural cavity 20 days after pneumonectomy Forceps points to area of bronchial venous covering

ing two dogs presented fistulas and were sacrificed for observation In both purulent liquid was found in the cavity When sacrificed the general condition of these dogs was very poor

**Examination of the Stump** In a gross study, the group where the stump was capped by vena cava grafts showed considerable firmness in the area of the stump after the third day This aspect was progressive during the following days and by the end of the tenth day had acquired a very consistent aspect The control group also tended to rapid firmness, but we believe that there was greater consistence in the first group (Figs 2a 2b, 2c)

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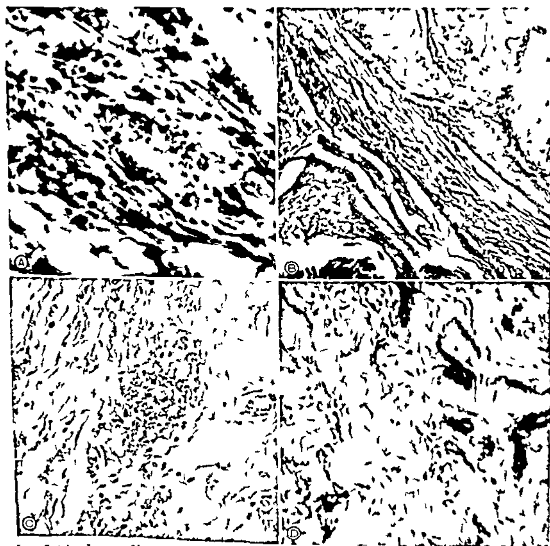



Fig 3 (a) Young fibroblastic exudation with inflammatory cells and capillary organization 48 days after bronchial venous covering 3 (b) Fibrous organization after 10 days 3 (c) Healing process at 60th day 3 (d) Conjunctive sclerosis at 105 days

The vein graft behaved as a foreign body which became encased by con junctive proliferating tissue on the internal part of the vein but which originated on the bronchi and the external part of the same portion of the vein where after the sixth day it acquired considerable firmness

A group of 12 human patients have been treated with this technique using pieces of human superior vena cava Postoperative control has passed the eighteenth month in one of them Observations to date have been satis factory and we hope in the future to be able to present the results of a con clusive number of cases

CONCLUSIONS

1 An experimental study in 200 dogs is reported In 100 animals pneu monectomy was performed capping the sutured bronchial stump with a frozen vena cava graft preserved at 8 to 10°C below zero The other 100 dogs were used as a control group

2 The mortality and morbidity were considerably lower in the group where the stump was capped by the vena cava graft

3 Histologic studies post operatively demonstrated the existence of a good fibrous reaction which gave firmness and consistence to the bronchial stump

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# CARDIOPULMONARY RESERVE FIVE TO FIFTEEN YEARS FOLLOWING FIFTY PER CENT OR MORE REDUCTION OF LUNG VOLUME *

Robert W. Harrison, Walter Buehler, Richard G. Thompson, Edwin T. Long, Ralph Carlson, Bernard Charbon, and William L. Adams

Extensive pulmonary resection frequently results in marked alterations of cardiopulmonary function even though pulmonary capacity under normal conditions has an enormous reserve. In certain individuals, notably those in the older age group, the cardiorespiratory system is unable to compensate adequately to pneumonectomy and death occurs in the early postoperative period.[1] Another group of patients undergoing extensive resection therapy is able to compensate sufficiently to the immediate effects of the sudden reduction in pulmonary parenchyma only to develop incapacitating symptoms of reduced cardiopulmonary reserve at a later time. Frequently these individuals are entirely asymptomatic at rest only to experience severe dyspnea on slight exertion. The "late" effects experienced by these individuals are not readily attributable to a lack of functioning lung tissue but are more likely due to a pulmonary vascular insufficiency with pulmonary arterial hypertension. The "late" effects of pneumonectomy will be of ever increasing importance with its widespread use today as the operation of choice for carcinoma of the lung.

A marked decrease in the amount of functioning lung in normal dogs produces alterations in the right ventricular pressures. A study from this laboratory showed that a 75 to 85 per cent reduction of vascular bed by resection of lung tissue in dogs resulted in immediate and sustained elevation of right ventricular pressure of the order of 90 per cent.[2, 3] In an attempt to better understand the mechanisms involved in the "late" effects of reduced lung volume, a group of patients and animals that had undergone extensive resection of lung tissue 5 to 15 yr previously were studied with respect to their *cardiopulmonary responses at rest and during exercise.*

## METHOD

Six dogs that had experienced 50 to 75 per cent reduction in functional lung volume 6 or more years previously were studied. Three of these animals had undergone staged pulmonary resections resulting in only the right upper and middle lobes remaining. The other 3 animals had their lung volume reduced by a combination of resection and the production of bronchial stenosis and resultant atelectasis.

Right ventricular or pulmonary artery catheterization was performed under local anesthesia as was cannulation of the previously transplanted subcutaneous carotid artery. Strain gauge manometers were employed for pressure determinations. A Wood cuvette with recording oximeter was attached to the cardiac catheter and carotid cannula allowing the periodic withdrawal

* From the Department of Surgery, University of Chicago Clinics, Chicago, Illinois. This investigation supported in part by a research grant (H 1894) from the National Heart Institute of the National Institutes of Health, Public Health Service and by grants from the Douglas Smith Foundation for Medical Research of the University of Chicago.

of blood samples for the determination of arterial and mixed venous oxygen saturations The dogs were exercised by requiring them to run on a tread mill at a speed of 1 75 miles per hour for periods of 2 to 5 min

Seven patients who had undergone pneumonectomy 5 to 15 yr previously were investigated They ranged in age from 45 to 73 yr Each patient was evaluated clinically with regard to his present occupation and activities in an attempt to classify present exercise tolerance

Functional capacity was graded on a scale of I to IV Class I included those patients having no limitation of physical activity and ordinary physical activity causing no discomfort Class II signified a slight to moderate limitation of physical activity, Class III a moderate to great limitation of physical activity while Class IV was assigned to the patients unable to carry on any physical activity without discomfort

Right heart catheterization was performed on each patient for measurement of right ventricular or pulmonary artery pressures Arterial oxygen saturation was determined by means of the Wood earpiece and recording oximeter[4] Mild to moderate exercise was accomplished by the patient bicycling against resistance while supine The duration of the exercise period was 1 to 4 min depending upon the abilities of the patient Observations of alterations in right heart pressure systemic blood pressure arterial oxygen saturation and pulse rate were made during the period of exercise

RESULTS

**Animals** The 3 animals that had undergone 75 per cent resection of lung tissue have been observed periodically with respect to right heart pressures In all cases there was an immediate marked increase in pulmonary artery pressure over the preoperative control level of the order of 67 to 188 per cent Over the course of 6 to 7 years the pulmonary artery pressures have gradually decreased to a level only 2 to 10 mm Hg above the preoperative levels Nevertheless all of the animals with markedly reduced functional lung volume of long standing when subjected to exercise showed a 36 to 84 per cent increase in pulmonary artery pressure over resting levels with an average increase of 64 per cent for the group The long term pulmonary artery pressure studies as well as the response to exercise are represented graphically in Figure 1

In general the alterations in arterial oxygen saturation were less striking with an average decrease of 3 per cent The systemic blood pressure and pulse rates showed increases of a variable degree during the exercise period

**Patients** Six of the patients were working full time in relatively sedentary occupations The other patient J S complained of slight dyspnea at rest and approximately 3 mo previously had been in frank congestive heart failure Three of the patients denied any dyspnea on ordinary activity while the other 4 described varying degrees of exertional dyspnea

Two patients had resting pulmonary artery pressures within the range of normal The resting pulmonary artery pressures in 3 others were slightly elevated (25 per cent) while in the remaining two patients the pulmonary artery pressures were increased two and threefold over the normal value at rest

In every patient the pulmonary artery pressure rose with the beginning of exercise and was sustained throughout the exercise period With the ces

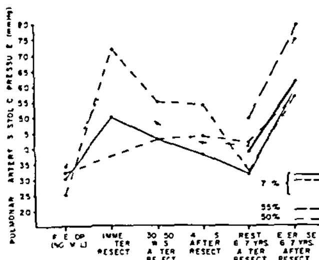



Fig 1 Pulmonary artery systolic pressures in dogs following a 50 to 75% reduction in functional lung volume All pressures except the last column were measured in awake resting animals The "exercise" pressures were measured while the animals were running on a treadmill at 1 75 mi /hr The heavy dark line connecting the last 2 columns illustrates the average pulmonary artery pressure response of the 6 animals

sation of exercise the right heart pressure showed a gradual fall but usually did not return to pre-exercise levels within 10 to 15 min The rise in pulmonary artery pressures was 15 to 49 mm Hg representing 45 to 140 per cent increases over the resting levels The average increase in right heart pressure for the group of patients was 29 mm Hg representing a 72 per cent increase over the average resting value of 40 mm Hg

The resting arterial oxygen saturation was normal to only slightly depressed The effect of exercise was in general less striking than in the case of the pulmonary artery pressures but in general (6 of 7 cases) the saturation decreased during the exercise period This decrease ranged from 2 to 14 per cent with an average of 5 per cent for the group The individual alterations in pulmonary artery systolic pressure and arterial oxygen saturation are summarized graphically in Figure 2 The systemic blood pressure and pulse rate increased during the exercise period as would be anticipated

## DISCUSSION

The pulmonary hypertension developing in animals as a result of marked reduction in lung volume gradually tends to return toward normal levels over the course of 6 to 7 years These compensatory changes in the pulmonary vascular bed however are not of such a nature or degree as to allow a normal response to exercise i e slight to no rise in pulmonary artery pressure

The patients studied revealed a fairly close correlation between their functional classification and the level of pulmonary artery pressure at rest and its alteration during moderate exercise No correlation was apparent between the patient s age or duration of reduced lung volume and his functional class or pulmonary artery pressures However it is our impression that pulmonary hypertension is more significant in the older age group of pneumonectomy patients

The normal cardiopulmonary response to exercise is one directed toward increasing the amount of oxygen uptake in the lungs to fulfill the increasing oxygen demand of the body tissues The oxygen uptake in the

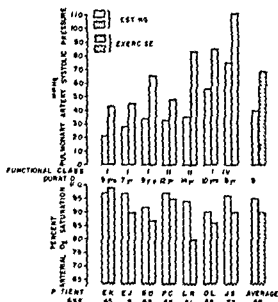



Fig 2 Comparison of the effect of exercise on pulmonary artery systolic pressures and arterial oxygen saturations in post pneumonectomy patients (arranged according to functional class — see text) Exercise consisted of moving the legs against resistance in the supine position

lungs may be increased either through increases in cardiac output or arterial venous oxygen differences In normal individuals during light exercise the increase in cardiac output greatly predominates and the change in arterial venous oxygen difference is minimized Exercise in the normal individual may increase cardiac output 2 to 2½ times with a slight elevation of systemic blood pressure while the right heart pressure and arterial oxygen saturation remain essentially unaltered [6]

The pulmonary artery pressure represents a summation of the left atrial pressure and the pressure gradient between the right ventricle and left atrium This gradient by Poiseuille's law, is directly proportional to cardiac output and inversely proportional to peripheral resistance a factor dependent primarily upon the number and caliber of vessels in the pulmonary bed

To account for the essentially unchanged right heart pressure in the normal individual during exercise with an increase in cardiac output, the pulmonary arterial resistance must be decreased by an increase in pulmonary vascular volume Riley *et al* [6] found that the pulmonary vascular resistance decreased to approximately one third of its resting value during severe exercise The reduced pulmonary vascular volume in the post pneumonectomy patient apparently often requires a higher than normal pressure gradient to maintain a normal rate of blood flow Furthermore this reduced pulmonary vascular bed is less able to accommodate the increased blood flow accompanying exercise thereby imparting increased vascular resistance and leading to an elevation of the right heart pressure It is not clear whether there is a fixed and restricted pulmonary vascular capacity in this situation resulting from simple volume reduction and anatomical arterial and arteriolar sclerosis or if there is in addition a reflex vascular change The latter factor has not been elucidated as yet so no therapy can be considered for it In recognizing the former factor the most valuable therapy is limitation

of extent of resection whenever feasible and instruction of the patient in restriction of his activities.

## SUMMARY


1. Resting right heart pressures of dogs with a 50 to 75 per cent reduction in functional lung volume show an average immediate increase of 90 per cent which tends to decrease over the course of 6 to 7 years to levels near normal. However, moderate exercise of these animals produces an average increase of 61 per cent in the pulmonary artery pressure.

2. Patients 5 to 15 years post pneumonectomy have a functional capacity that correlates with the degree of pulmonary hypertension. Resting pulmonary artery systolic pressures average 40 mm. Hg and increase an average of 72 per cent during moderate exercise. Exercise usually results in a decrease in peripheral arterial oxygen saturation.


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# A STUDY OF THE BRONCHIAL ARTERY FLOW IN THE DOG*

David State, Peter F. Salisbury and Peter Weil

Anatomical studies have demonstrated dilatation of the bronchial vessels in congenital heart disease and in acquired pulmonary lesions.[1, 2, 3] The observed morphological changes parallel alterations of bronchial flow; in certain congenital cardiac abnormalities the bronchial arteries may carry up to 50 per cent of the cardiac output.[3] The flow in isolated bronchial arteries has been measured with flow meters,[4] and there have been attempts to measure the total bronchial flow[5, 6] in isolated preparations. The development of effective and manageable equipment for total body perfusion has afforded us the opportunity to measure bronchial flow by a technique utilizing separate perfusions of the greater and lesser circulation.

## METHOD

The acute experiments described here were performed on 14 mongrel dogs, 8 to 30 kg in weight. They were anesthetized with pentobarbital sodium, 5 mg/kg, and given heparin, 2 mg/kg, intravenously. Pressures in the femoral artery and in a branch of the pulmonary artery were registered with plastic cannulae connected to strain gauges, amplifiers and a multi-channel direct writing oscillograph. The heart and great vessels were exposed under positive pressure endotracheal respiration by a bilateral trans-sternal thoracotomy through both 4th intercostal spaces. A plastic tube (Bardic No. 21 French) for the withdrawal of venous blood was inserted into the right ventricle through the right auricular appendage. When a ligature about the pulmonary artery was tied, all venous blood from the right heart was then diverted through the heart-lung machine. All blood from the left ventricle was drained into a graduate reservoir which was placed 10 cm below the dog's spine. Blood from this reservoir was re-injected by a variable speed Sigmamotor pump into the pulmonary artery. With this arrangement blood

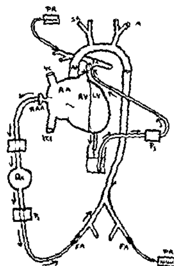



Fig. 1. System for independent perfusion of the systemic and pulmonary circulation. RAA, right auricular appendage; RA, right atrium; RV, right ventricle; LV, left ventricle; VCS, vena cava superior; VCI, vena cava inferior; Ao, aorta; PA, pulmonary artery; SA, subclavian artery; CA, carotid artery; FA, femoral artery; $P_{1,2,3}$, pumps; Ox, oxygenator; RES, reservoir; PR, pressure recordings. Arrows indicate direction of blood flow. Changes in reservoir level indicate changes in bronchial artery flow.

*From the Department of Surgery and the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, California. Supported by a grant from the Los Angeles County Tuberculosis and Health Association.

which issued from the pulmonary veins could only come from the bronchial arteries or from blood pumped into the pulmonary artery. Since the latter drains into the reservoir, any rise in blood level in this collecting chamber must be due to bronchial flow. The quantity of this flow per standard observation period can thus be read directly from the blood level in the reservoir.

The systemic circulation of the animals was perfused with arterial flows ranging from 25 cc./kg./min. to 70 cc./kg./min. This resulted in systemic arterial pressures ranging from 40 to 110 mm. Hg systolic. Pressures in excess of this were observed after administration of phenylephrine hydrochloride (neosynephrine (R)). Blood was perfused into the arterial tree via the femoral or subclavian artery with a distinct pulsatile quality. Back leakage of the blood through the aortic valve was prevented, when necessary, by a Blalock clamp on the ascending aorta.

A short period of perfusion of both circulations was necessary to obtain a steady state before observations were made.

**Effect of Variation of Systemic Arterial Pressure Upon Bronchial Artery Flow.** The immediate increase and decrease of bronchial flow with corresponding changes of the systemic pressure was one of the most consistent and reproducible findings in this series of experiments. When the systemic arterial pressure is kept constant (with small amount of hexamethonium) while the arterial perfusion is increased, increased flow in the peripheral arteries did not cause a corresponding increase of flow in the bronchial vessels; this indicates that the systemic pressure and not the systemic flow is the effective agent which influences bronchial flow. At systolic arterial pressures of 90 to 100 mm. Hg the average flow in the bronchial arteries was equal to 0.5 to 1.0 per cent of the total arterial blood flow.

**Effect of Variation of Pulmonary Artery Perfusion upon Bronchial Artery Flow.** When other factors were kept constant but the flow in the pulmonary circuit was varied, consistently reproducible changes were not observed. In the main, bronchial flow remained constant regardless of the increase, decrease, or even complete arrest of the pulmonary perfusion as long as the p.a. flow did not exceed 50 ml./kg./min. When the pulmonary perfusion was arrested, the partition of bronchial flow between the pulmonary artery and the left atrium was variable and a consistent pattern was not observed.

*Table 1. Effect of Variation of Systemic Arterial Pressure on Bronchial Artery Flow*

| DOG NO. | WEIGHT IN KG. | SYSTOLIC FEMORAL ARTERY PRESSURE FROM | TO | RISE OR FALL EFFECTED BY | BRONCHIAL FLOW IN ML./MIN. FROM | TO |
|---|---|---|---|---|---|---|
| 264 | 20 | 40 | 55 | Flow through pump | 2 | 5 |
| | | 55 | 30 | | 6 | 2 |
| " | | 30 | 50 | | 2 | 5 |
| | | 50 | 65 | | 5 | 10 |
| | | 65 | 35 | | 10 | 4 |
| 266 | 24 | 70 | 160 | Neosynephrine | 8 | 27 |
| " | | 160 | 140 | After neosynephrine | 22 | 8 |
| " | | 140 | 170 | Neosynephrine | 8 | 15 |

*Table 2 Effect of Variations of Pulmonary Artery Flow upon Bronchial Artery Flow*

| DOG | WEIGHT IN KG | PULMONARY ARTERY FLOW IN ML/MIN FROM | TO | BRONCHIAL FLOW IN ML/MIN FROM | TO | PULMONARY ARTERY PRESSURE FROM | TO | SYSTOLIC FEMORAL ARTERY PRESSURE |
|---|---|---|---|---|---|---|---|---|
| 264 | 20 | | 220 | 5 | 5 | 0 | 6 | 55 |
| | | 220 | 530 | 5 | 5 | 6 | 20 | 55 |
| | | 530 | | 5 | 6 | 20 | 0 | 55 |
| | | | 220 | 6 | 6 | 0 | 9 | 55 |
| | | 220 | 100 | 2 | 2 | 9 | 16 | 30 |
| | | 100 | – | 2 | 2 | 16 | 0 | 30 |
| | | | 220 | 10 | 10 | 10 | 14 | 65 |

**Effect of Respiratory Volumes on Bronchial Artery Flow** In 5 experiments deflation of the lungs and arrest of positive pressure respiration caused a decrease of bronchial flow of up to 60 per cent of the control value In 1 additional experiment no changes were observed Upon resumption of ventilation the bronchial flow increased in 4 out of 5 observations In these experiments the systemic arterial pressure did not change significantly

**Effect of Hypercapnia and Acidosis on Bronchial Flow** In 7 experiments total body perfusion was maintained but the ventilation of the independently perfused lungs was temporarily effected by 10 per cent $CO_2$ and 90 per cent oxygen The pulmonary perfusion blood was routed through a loop which contained glass electrodes connected to a continuously registering pH meter When 10 per cent $CO_2$ in oxygen was used, a rapid fall of pH of the blood in the pulmonary circuit occurred In 5 out of 6 experiments when the systemic arterial pressure remained constant a change of inhalation gases from 100 per cent oxygen to 10 per cent $CO_2$ and 90 per cent oxygen resulted in an increase of bronchial artery flow up to 160 per cent above control values

**Effect of Serotonin (5 hydroxytryptamine) on Bronchial Flow** Addition of 10 mg of serotonin creatine sulfate to the blood in the oxygenator caused a sustained pronounced increase in bronchial artery flow averaging 160 per cent above controlled values in 5 experiments After serotonin the bronchial flow was elevated for periods of up to 60 min and the increased flow diminished only gradually Repeated administration of serotonin caused further increases of bronchial flow After the administration of serotonin the well known pronounced rise in pulmonary artery pressure was observed but systemic arterial pressure remained the same or dropped slightly

DISCUSSION

The dual circulation in the lungs has been investigated extensively with anatomical methods the results of such studies have suggested a potential role of the bronchial vascular system in the pathogenesis and the pathological physiology of cardio pulmonary disease There have been several indirect and partial measurements of flow in the bronchial artery territory but the only attempt to measure total bronchial artery flow directly was performed in 1932 by Berry and Daly who perfused the pulmonary and the bronchial circuits of isolated lungs lung thorax and lung esophagus preparations As far as we know the total collateral blood flow to the lungs has

not previously been measured in animals with intact reflexes

When the lesser circulation is perfused separately from the systemic circulation any increase of the blood volume in the lesser circulation must come from the bronchial arteries The term bronchial artery flow as used in this article applies only to that portion of bronchial artery flow which drains into the pulmonary veins It is known that some collaterals exist between bronchial and azygos veins as well as the superior vena cava[8] The amount that flows in these channels is not known but is felt to be insignificant unless chronic obstructions in the pulmonary veins occur


## SUMMARY

1 A method has been developed for measuring bronchial arterial flow by a technique utilizing independent perfusions of the systemic and pulmonary circulation

2 The bronchial artery flow is directly related to systemic arterial pressure With normal blood pressures the anastomotic channels between bronchial arteries and pulmonary vein carry 0 5 to 1 0 per cent of the total circulating blood volume per minute

3 Deflation of the lungs causes a decrease in bronchial arterial flow ranging up to 60 per cent of control values

4 The use of 10 per cent $CO_2$ in oxygen to ventilate the lung (but not the pump oxygenator) increases bronchial flow markedly over control values when 100 per cent oxygen is used

5 Changes in pulmonary artery flow rate did not consistently affect the flow in the bronchial arteries when p a flow was less than 30 ml /kg/min

6 Administration of serotonin (5 hydroxytryptamine) markedly increased bronchial flow


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# HUMAN MEASUREMENTS INVOLVED IN TRACHEOBRONCHIAL RESECTION RECONSTRUCTION AND PROSTHETIC REPLACEMENT A PRELIMINARY REPORT ON IVALON SPONGE*

JOHN E JESSEPH JOHN K STEVENSON HENRY N HARKINS AND K ALVIN MERENDINO

One facet of our approach to the general problem of tracheobronchial resection reconstruction and prosthetic replacement has dealt with a considerable body of anatomic data from the study of a group of fresh human cadavers At that time consideration was given to only a few of the more important dimensions of the trachea and main bronchi [8] More recently, the data have been re examined in an attempt to determine whether or not there is any more or less constant pattern of structure of the tracheobronchial tree If such were true one could then plan reconstructive and prosthetic procedures in advance

Consideration will be given here to some of the important anatomic considerations which relate to the development and application of tracheal prosthetics

## METHOD

**Anatomic Data** In the total of 136 bodies originally studied there were 56 (35 female 21 male) in which observations had been made in sufficient detail for a complete analysis Lengths and diameters of bronchi and their orifices down to and including the tertiary (segmental) bronchial orifices were available In studying the over all distribution of these dimensions and in attempting to correlate them with other parameters such as sex height race etc a considerable mass of tabular data was accumulated These findings will be published in detail elsewhere

In the study of the 56 bodies several relationships were noted to be of fair constancy The human male trachea has a mean luminal diameter of 2 2 cm the female of 1 7 cm The ranges of each are 1 8 to 2 5 cm and 1 4 to 2 0 cm respectively All measurements were made just above the bifurcation If the luminal diameters of the right and left main bronchi are compared by ratio i e RMB LMB it is found that for males the ratio is 1 2 1 $\pm$ 0 1 For females the ratio is also 1 2 1 $\pm$ 0 2 Further, it is found that the total cross sectional area of the two main bronchi almost exactly equals the cross sectional area of the trachea for both sexes Thus it can be said that the square of the radius of the trachea is approximately equal to the sum of the squares of the radii of the main bronchi This relationship is subject to a variation of only 7 per cent which for purposes of surgical reconstruction is a negligible error

**Tracheobronchial Prosthetic Replacement** A number of investigators have experimentally explored several methods of prosthetic replacement of excised segments of the trachea and main bronchi [1 2 4 9] A few applications of such artificial tubular replacements have been made in humans but with

*From the Department of Surgery University of Washington School of Medicine Seattle Washington Supported in part by funds from an Institutional Grant from the American Cancer Society

rare exceptions no method has provided even semi permanent restoration of function [3-6]

Probably the greatest single problem in tracheal replacement is the method of securing union between the ends of the host trachea and the prosthesis so as to ensure air seal and yet avoid necrosis of the tracheal stumps Stenosis secondary to granulation tissue ingrowth, loosening of the replacement with resultant tracheal collapse and obstruction as well as leakage with infection are all hazards to tracheal substitution With these adversities as a guide a new type of prosthesis was devised

**The Prosthesis** Ivalon (polyvinyl alcohol) sponge has the well known property of retaining a deformity imposed and maintained during heating Sheets of sponge can be fabricated into tubes plugs and cylinders of any desired size and form Compression renders the sponge less permeable and increases its strength and elasticity Vascular grafts thus formed have shown considerable experimental promise [7-10]

For tracheal replacement a cuffed tubular prosthesis is made by wrapping several thin (2 to 3 mm) layers of sponge about a smooth glass or metal rod Narrow rings of lucite (methyl methacrylate) are placed between the layers so that the tube has radial rigidity but retains some longitudinal flexibility At each end of the tube and likewise between the sponge layers are placed lucite rings of about 7 mm width to provide a seat for the points of the fixation ring The sponge layers over this latter lucite ring are separated by interposing several layers of cotton tape The final result is a cuff which can be retracted to expose each end of the prothesis and its terminal ring (Fig 1) Final form is achieved by wrapping boiling and cooling

The method of fixation of the prosthesis is a modification of Hufnagel s multiple point concept Stainless steel bands are used each having several sharp pointed pegs which pierce the tracheal wall and impinge upon the tubes terminal ring preventing it from slipping out of the tracheal lumen The ends of the fixation bands are secured by a single twist of stainless steel wire This method does not rely on simple pressure transmitted through the wall for fixation and is consequently less likely to produce necrosis of the tracheal wall

**Experimental Application** Adult healthy mongrel dogs of either sex were used Under Nembutal anesthesia a high right thoracotomy was performed under sterile conditions The azygos vein was ligated and divided and the trachea circumferentially dissected free of all attachments from the carina

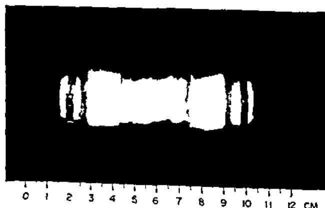



Fig 1 The Ivalon sponge tracheal prosthesis The cuffs are retracted to show the stainless steel fixation rings which hold the prosthesis in place within the trachea

upward for a distance of about 8 to 10 cm. The trachea was then divided about 2 cm above the carina and the left main bronchus intubated so that ventilation could be maintained during placement of the prosthesis. A segment of trachea averaging 4 to 6 cm in length was excised. A prosthesis of suitable diameter was then chosen, the end cuffs retracted, and one end inserted into the proximal tracheal stump. The fixation ring was then tightened about the trachea, so that its points passed through the wall and were firmly imbedded into the terminal lucite ring. The band was held in position by twisting a loop of #28 stainless steel wire passed through holes at either end. The opposite end of the prosthesis was similarly fixed into the distal tracheal stump after withdrawal of the endobronchial tube. Placing and fixing the prosthesis required only a few seconds, so that there was no significant interruption of ventilation. Finally the cuffs of the prosthesis were drawn over the metal fixation rings, protecting the contiguous mediastinal structures as well as providing a complete air seal (Fig. 2). Postoperatively all dogs were given antibiotics and fed a soft diet.

## RESULTS

A total of 14 dogs were subjected to excision and substitution of tracheal segments as described. Of these 10 have survived and carry on all normal canine functions, the longest after an interval of 10 months. Two deaths occurred in the immediate postoperative period, 1 from a massive air leak through an unrecognized defect in the prosthesis, the other from empyema. Two late deaths (after 2 mo.) resulted from the prosthesis slipping out of the trachea with subsequent intratracheal granulation tissue formation, obstruction, and pneumonia. These occurred early in the series, and it was found that the points of the fixation bands were too short and had not imbedded deeply enough into the prosthesis.

Each dog has been examined by serial bronchoscopy beginning in the first postoperative week. At 6 months after transplantation the trachea has a normal appearance without granulations, and the prosthesis continues to fit snugly into the lumen. There is no apparent retention of secretions either in or distal to the prosthesis itself, although the prosthesis reduces the lumen 2 to 1 mm—the magnitude of its wall thickness.

Bifurcated prostheses have recently been made for replacement of the distal trachea, carina, and the proximal portions of both main bronchi. As yet an insufficient number of these have been placed to allow any conclusions.



Fig. 2. Operative view of an Ivalon sponge tracheal prosthesis in place with cuffs pulled over the fixation rings.

## SUMMARY

Data obtained from the study of fresh human cadavers indicates that within certain limits it is possible to predict the dimensions of certain parts of the upper tracheo bronchial tree This is based on the finding of a considerable relative constancy of dimension among these parts

A new tracheal prosthesis is described and some preliminary observations made following its application in dogs A 9 mo follow up shows a high rate of survival and an excellent maintenance of airway and pulmonary function


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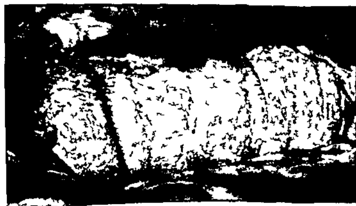


Fig 2. Operative view of an Ivalon sponge tracheal prosthesis in place with cuffs pulled over the fixation rings.

studies of the heart were done after perfusion of the coronary arteries with Diodrast 4 The right ventricle was distended with fluid to determine the pressure necessary to rupture the wound 5 Strips of myocardium cut transversely through the wound and measuring 1 cm in width were tested for disruptive strength

### RESULTS

The electrocardiographic examinations were for the most part within normal limits In 2 dogs there was evidence of slight myocardial ischemia probably associated with the anesthesia In none of the dogs with the myocardial thinning and fibrosis was there an abnormal tracing In 1 dog with pulmonary artery stenosis there was right axis deviation the others in this group were essentially normal

In all animals some degree of adhesive pericarditis was present especially over the scar None had aneurysms of the ventricle nor was there a discernible alteration in heart action In the 2 in which a clamp was utilized scarring through the full thickness of the myocardial wall occurred along the site of application

Of important significance were areas of definite myocardial thinning between the wound and the anterior descending branch of the left coronary artery in 6 animals These areas varied in size from 2 5 cm x 1 5 cm to 0 8 x 0 4 cm This tissue appeared to be entirely fibrous in nature No thrombi were present on the endocardial surface sutures were endothelialized No abscesses could be identified but in 3 dogs there was extreme tissue reaction about the sutures

Histologically healing was characterized by dense collagen in and around the wound edges An unexpected finding was severe chronic inflammatory response around the silk sutures in several dogs In 1 animal with grossly discernible abnormal tissue about the sutures the reaction was very severe even though the operation had been performed 10 months previously

The grossly demonstrated areas of marked thinning of the ventricular wall showed almost complete absence of myocardium histologically The wall consisted of fibrous tissue which appeared to originate from the epicardium and endocardium accompanied by considerable fat infiltration In some areas there were a few scattered bundles of myocardium

Visualization of the coronary arteries by the injection of Diodrast was attempted in 3 dogs and in 2 there were satisfactory films There was no abnormality of the larger vessels In 1 dog with satisfactory postoperative healing the opacification of the myocardium on the side of the wound toward the left coronary artery system seemed less dense than in normal animals This is the area in which poor healing occurred in several dogs

In several hearts all vessels except the pulmonary artery were occluded with clamps and the right ventricle was distended with fluid Heart valves became incompetent or the occluded vessels ruptured but the ventricular scar remained intact although an intraventricular pressure of over 250 mm Hg was reached in 2 Continuance of this test seemed to be of little value and disruptive studies were performed on the myocardial tissue in those remaining

A method utilizing constantly increasing tension applied to the ends of a 1 cm strip of myocardium cut transversely through the scar was used to

# *The Heart Valvular Disease, Open Heart Surgery, Pump Oxygenator and Cardiac Arrest*

## STUDIES IN HEALING OF LARGE RIGHT VENTRICULOTOMIES*

WILLIAM H MULLER JR, W DEAN WARREN J FRANCIS DAMMANN JR AND WILLIAM H MARSH

Further developments and refinements of extracorporeal heart lung systems have made possible the correction of intracardiac defects which heretofore were not amenable to corrective surgery The healing of auricular wounds has presented no problem However, the high intraventricular pressure and the fact that the ventricular wall is chiefly muscle make the ultimate fate of large ventriculotomies a potential problem [1] It seemed timely therefore to investigate by various techniques certain aspects of healing of large ventricular wounds

### METHOD

Twenty mongrel dogs weighing 11 to 18 kg were used Intravenous nembutal (12 mg /lb ) was employed for anesthesia In 2 experiments the left pleural cavity was entered through the fourth intercostal space The heart was exposed through a large incision in the pericardium and a traction suture was placed in the midportion of the right ventricle By placing traction on this suture a large portion of the right ventricle wall was grasped with a curved non crushing vascular clamp and a longitudinal incision 3 to 4 cm long was made through the full thickness The edges were then approximated with a continuous 3 0 atraumatic silk suture placed 3 mm apart through the full thickness 5 mm from the cut edge The pericardium was reapproximated with interrupted silk sutures and the chest was closed in the *routine fashion employing water seal drainage Subsequently* 18 animals were perfused with a heart lung system and right ventriculotomies measuring 5 to 6 cm in length were made The myocardial wounds were closed in the manner previously described In 5 animals pulmonary artery stenosis was created after the ventriculotomy was made to increase the right ventricular pressure and place the wound under greater stress [2] Penicillin 600,000 units was given at the conclusion of the procedure The dogs were sacrificed at intervals ranging from 2 to 16 mo

The following studies were performed 1 Electrocardiographic tracings were obtained before and at intervals after the ventriculotomies 2 Immediately prior to sacrifice a thoracotomy was performed and the heart action was grossly observed In those animals having artificially created pulmonary artery stenosis pressures were measured in the right ventricle and pulmonary artery Gross and histologic examinations of the healed scar and surrounding myocardium were made of autopsy specimens 3 Roentgenologic

*From the Department of Surgery University School of Medicine Charlottesville Virginia Supported by U S Public Health Grant #2038 for Studies on Problems Relating to the Surgical Treatment of Acquired and Congenital Heart Disease

CONCLUSIONS

This study demonstrated no complications associated with the myocardial thinning which occurred in 6 or 30 per cent of the animals Although these data cannot be translated directly to cardiac wound healing in patients the defects in the myocardium must be considered a potential source of future difficulty The problem of the healing of ventriculotomy wounds warrants further investigation

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# THE USE OF ALCOHOL IN THE TREATMENT OF THE POSTVALVULOTOMY HYPONATREMIC SYNDROME*

GEORGE J D ANGELO H V MURDAUGH JR WILL C SEALY AND IVAN W BROWN JR.

One of the complications following mitral commissurotomy is the syndrome of oliguria water retention hyponatremia and hypochloremia as described by Wilson *et al*[13] and Goodyer and Glenn[4] This syndrome although frequently transient can become a problem in the postoperative management of seriously ill patients Goodyer[4] suggested the possibility of an antidiuretic stimulus with the hyponatremia resulting from hemodilution as in postoperative oliguria[7]

Leaf and Mamby[6] reported an abnormal response to water loading in dogs made hyponatremic by dialysis In normal dogs measurable antidiuretic activity of the plasma disappeared with water loading and diuresis occurred In the hyponatremic dogs antidiuretic activity of the plasma was still detected following water loading and diuresis did not occur even though hemodilution was produced

In the study of patients with the postvalvulotomy hyponatremic syndrome it was noted that they had water retention with concentrated urine of low volume in the face of electrolyte dilution as evidenced by the low serum sodium and decreased plasma osmolarity The similarity of this condition and that produced by excessive doses of pitressin together with the observations made by Leaf[6] led to the inquiry of the possible role of the antidiuretic hormone in hyponatremic states Since alcohol reportedly inhibits the

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determine the disruptive strength Duplicate strips were taken from each specimen and an average was obtained In no instance did the healed wound disrupt In every instance including 5 hearts with gross thinning adjacent to the wound the site of disruption was through normal myocardium or at a point of attachment of the myocardial strips in spite of special precautions in attaching the strips to the tension sources

In the disruptive strength studies an average tension of 1650 gm could be tolerated before disruption occurred In the 12 hearts tested this ranged from 2100 gm to 900 gm



Fig 1 A well healed ventriculotomy wound viewed through the opened right ventricle Note the normal myocardium on either side of the incision



Fig 2 Healed ventriculotomy wound created by the clamp method The inner well healed scar is the incision and the broad lateral scars are from the blades of the clamps

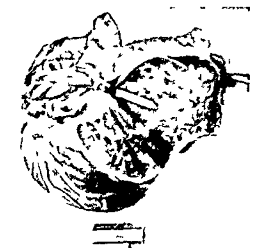


Fig 3 Ventriculotomy specimen in which a rather large area of thinning and fibrosis has occurred between the incision and the left coronary artery system Much of this area was found devoid of myocardial tissue on histologic examination

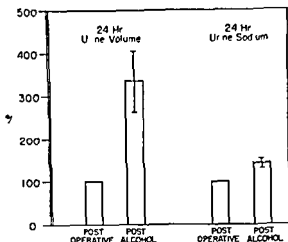



Fig 2 Diagram comparing the change in urine volume and urinary sodium output following alcohol therapy 100% represents the mean of the pre alcohol (oliguric) values

per volume fluid intake Following alcohol administration the increase in sodium output in the urine was much less than the increase in the urine volume (Fig 2)

The hyponatremia and the decrease in plasma osmolarity an expression of hemodilution which occurred during the early postoperative period was correctable by alcohol administration as depicted by Figure 3 Figure 4 represents an illustrative case demonstrating the described changes

With osmolarity determinations performed on the urine at frequent intervals dilution of the urine as evidenced by a decrease in urine osmolarity to as low as 130 mOsm/L occurred during the period of diuresis By collecting the urine samples at periodic intervals the decrease in urine concentration during diuresis is more readily demonstrated than by studying the 24 hr collection as one sample

## DISCUSSION

A transient disturbance in the metabolism of water and electrolytes is a common phenomenon during the immediate postoperative period However in some cardiac patients these changes are exaggerated[1] and present a clinical problem It is this group of patients that develop the hyponatremic syndrome with oliguria water retention and a concentrated urine in the face of hemodilution To correct this metabolic imbalance the use of alcohol has been effective

The patients studied developed hyponatremia and hypochloremia between the second and fourth postoperative days Those patients followed

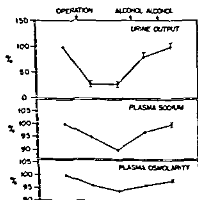



Fig 3 Diagram demonstrating the electrolyte dilution during oliguria and the correction that corresponds to the diuresis 100% represents preoperative values

release of antidiuretic hormone from the posterior pituitary[2, 5, 10, 11] and was reported to produce diuresis in the state of water intoxication[8] the use of alcohol in the treatment of postcommissurotomy hyponatermia was explored

## METHOD

Serum and urine electrolyte measurements and plasma and urine osmolarity determinations were followed in all patients scheduled for mitral commissurotomy Daily fluid intake and output data were obtained When the hyponatremic syndrome occurred the patient was given 20 to 50 ml of absolute alcohol on the 2nd 3rd or 4th postoperative day The alcohol was given in fruit juice by mouth or if nausea was present it was given as a 5 per cent intravenous infusion in 5 per cent dextrose in water The postoperative fluid intake was maintained between 1500 and 2000 ml per day including the alcohol

Blood samples were analyzed for serum sodium potassium and chloride concentrations and plasma osmolarity The 24 hr urine collections were measured for specific gravity osmolarity and content of sodium and potassium When possible the osmolarity of the urine was measured every 2 to 4 hr

The patients were maintained on the same low salt diet that they received preoperatively No supplementary salt was given orally or parenterally

## RESULTS

Five patients of the 21 followed developed the postvalvulotomy hyponatremic syndrome In these 5 patients there was a decrease of 7 to 20 mEq/L in the serum sodium concentration and a decrease of 6 to 20 mOsm/L in the plasma osmolarity from their preoperative values Their urine was concentrated and the volume was less than 500 ml per 24 hr period Following alcohol administration a diuresis occurred with the 24 hr urine volume being 2 to 6 times the pre alcohol 24 hr volume The postoperative oliguria and the response to alcohol administration are graphically demonstrated in Figure 1 The *ad libitum* preoperative fluid intake was greater than the fluid intake allowed after surgery Thus the increase in urine 24 hr volume to 100 per cent of the preoperative value represents a greater urine output

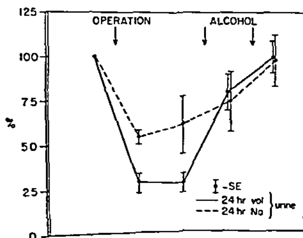



Fig 1 Diagrammatic illustration of the postoperative oliguria and response to alcohol 100% represents the preoperative value Each point depicted between operation and alcohol therapy represents the mean value for one half the interval between surgery and therapy (2 to 4 days) to demonstrate the constancy of the oliguric state

(20 to 50 ml) there was a marked increase in the urinary output with a return of the plasma osmolarity, serum sodium and serum chloride to normal. Studies of urinary electrolyte excretion during the phase of increased urinary output suggests a true water diuresis with conservation of sodium. The possible mechanism of this phenomenon is discussed. These studies indicate that alcohol is an effective therapeutic tool in the management of the postcommissurotomy hyponatremic syndrome. The importance of fluid restriction following mitral surgery is stressed.

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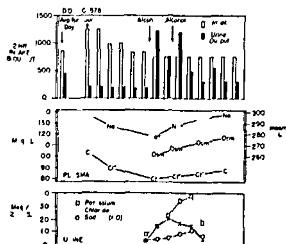



Fig. 1. A case illustrating the occurrence of the hyponatremic syndrome and recovery following alcohol therapy.

but not included in the data showed no tendency to develop this syndrome after the fourth postoperative day. In the patients developing the hyponatremic syndrome the administration of 20 to 50 ml. absolute alcohol produced a diuresis within 12 hr. The low sodium content of the large urine volume following alcohol administration indicated that the body was retaining sodium and losing water. In these patients the result of the diuresis was a return of serum sodium and chloride and plasma osmolarity to normal. The rise in the serum chloride concentration paralleled the change in serum sodium but at a slower rate of recovery. Clinical improvement in these patients was apparent immediately following their diuresis.

It is suggested that this effect of alcohol in inducing a diuresis is due to its inhibition of the release of antidiuretic hormone from the posterior pituitary. It is apparent that the inhibition of the posterior pituitary would have to be of high efficiency since van Dyke[1] has demonstrated that the human posterior pituitary contains about 15,000 mμ of antidiuretic hormone and that the human requires only 0.5 to 2.0 mμ to cause antidiuresis.

Gauer and associates[3] suggested that the oliguria produced by the positive pressure breathing experiments of Drury *et al.*[1] and the diuresis occurring during negative pressure breathing could be related to stretch receptors in the auricles and great veins within the thoracic cavity. He further postulated that the antidiuretic hormone could be involved in this phenomenon. Based on these experiments it has been suggested by Sieker[9] that hyponatremia following mitral valvulotomy may be related to reduction of pressure in the left auricle by the valvulotomy. This may explain why the electrolyte imbalance following mitral surgery is more pronounced than that seen after noncardiac surgery.

One of the most important features that has become apparent during this study is the necessity of fluid restriction following mitral surgery. Since our first patient was treated with alcohol there has been more rigid control of postoperative fluid intake. As a result there has been an apparent decrease in the frequency and severity of the syndrome.

## SUMMARY

Five postvalvulotomy patients had a concentrated urine of low volume, low serum sodium, low serum chloride and electrolyte dilution as evidenced by decreased plasma osmolarity. After the administration of absolute alcohol

It has been noted in the literature that a single silk strand strutted across the cavity of the left ventricle soon acquires a smooth endothelial like covering. With this in mind, the thought occurred that perhaps nylon would act in the same way, and such was the case. The problem of creating a valve like flap was met by fashioning a frame of Elgiloy and covering it with nylon.

Elgiloy* is an alloy developed by the Elgin National Watch Company that has several properties which make it ideal for this purpose. First, it is inert in the body; second, the characteristics of the alloy are such that it will maintain its spring properties for a period far exceeding normal human life expectancy; third, it can be shaped in a soft pliable state and with simple working and heating is made to acquire spring properties. The frame is made of flat stock which limits lateral motion of the prosthesis even though anchored at a single point. Thus directional motion is obtained with uni point fixation.

METHOD

In working out a technique for insertion, prostheses of various shapes were placed in the mitral area of 65 dogs and the aortic area of 45 dogs.

In the mitral, a malleable introducer is passed through the wall of the left



Fig. 2a. Four month mitral prosthesis.



Fig. 2b. 180X. Three month aortic prosthesis. The black braid is silk suture, the grey braid is nylon.

*Material and technical advice through the courtesy of Mr. Thomas R. Green, Elgin National Watch Co., Elgin, Ill.

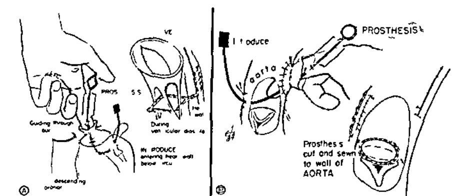
# *SPRING VALVE PROSTHESIS FOR THE CONTROL OF VALVULAR INSUFFICIENCY**

JAMES H WIBLE, LYLE F JACOBSON PRESCOTT JORDAN JR, AND CHARLES G JOHNSTON

In recent years many approaches to the surgical alleviation of valvular insufficiency have been tried Some have been used in selected clinical cases, some have never come to clinical trial, none have met general success The multitude of approaches attest to the lack of a satisfactory method of control

Despite the type of lesion valvular insufficiency results from the absence of effective coapting valvular tissue It has been our working premise that except for the occasional circumstance a prosthesis is necessary to cover this deficient area In order to create a workable device arbitrary criteria were set up which we felt should be met

A prosthesis should 1) control insufficiency 2) be easily inserted 3) move and continue motion 4) be self repairing i e grow living tissue 5) not obstruct the normal directional flow of blood 6) not embolize or form clot that can embolize 7) be tolerated within the circulation i e not extruded as a foreign body and 8) not alter blood elements including clotting mechanisms



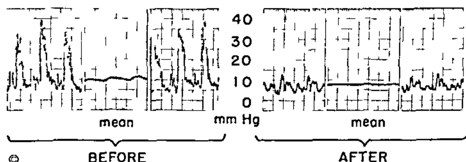




Fig 1a Technique for insertion of mitral prosthesis
Fig 1b Technique for insertion of aortic prosthesis
Fig 1c Left atrial pressures obtained by direct puncture before and after insertion of mitral prosthesis

*From the Department of Surgery Wayne State University College of Medicine Detroit Receiving *Hospital and the Dearborn Veterans Administration Hospital Aided by a grant* from the Michigan Heart Association the Research Corporation of the Detroit Receiving Hospital and the Veterans Administration Hospital

# EXPERIMENTAL PROBLEMS IN THE CONSTRUCTION OF SUBCORONARY PROSTHETIC AORTIC VALVES*

W. G. Malette, W. B. Summers and B. Eiseman

The definitive surgical correction of aortic valvular disease awaits the development of a suitable prosthetic valve that can be inserted beneath the coronary ostia. Valves placed in the aorta distal to the coronary ostia prevent regurgitation of a part of the cardiac output and thereby partially relieve the increased left ventricular strain, but only if placed beneath the coronary ostia can they serve the other function of the aortic valve—namely, to divert aortic blood into the coronaries during cardiac diastole.

Although a completely successful valve has not yet been achieved, a report of experience in the design and insertion of subcoronary prosthetic aortic valves in 53 dogs seems warranted in order to define the technical problem and to describe various procedures that have been employed in its attempted solution.

## METHOD

**Operative Exposure.** Surgical exposure of the aortic valve is possible either through the left ventricle or via an incision in the base of the aorta.

In 7 animals various modifications of the left transventricular approach were utilized, but exposure of the annulus was inadequate due to limitation of the incision by descending branches of the left coronary artery. This approach was therefore abandoned.

A more direct and satisfactory approach to the aortic valve is via a longitudinal incision in the base of the ascending aorta, as has been described by others.[1, 2, 3] Certain anatomic features of this area are of peculiar importance in the placement of subcoronary prosthetics and bear emphasis. The root of the aorta is a friable, easily torn structure that lies buried in the myocardium and is surrounded by vital structures. It is best approached by retracting the right atrium laterally, thus exposing the right anterolateral surface *of the aorta where an aortotomy incision can be extended to the* annulus without involving adjacent structures. Elsewhere about the circumference of the base of the aorta longitudinal incisions are limited inferiorly by myocardium.

By placing tension on 2 traction sutures placed in the annulus, good exposure of the entire area is obtained. The tissues immediately above and below the annulus are friable so that sutures must be placed accurately only in the annulus, which is firm, elastic and holds stitches well. On every side lie vital structures that must not be enclosed with sutures.

**Perfusion.** In 4 animals hypothermia (27°C.) with cardiac inflow-outflow occlusion has been utilized[4] but excision and meticulous replacement of the aortic valve with a prosthetic substitute requires a more prolonged period of open cardiotomy than is thus provided. Cross clamping of the ascending aorta and body perfusion with oxygenated blood is therefore required. In 3 cases reservoir oxygenated blood was perfused above the clamped aorta

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*From the Department of Surgery, University of Colorado School of Medicine and the Denver Veterans Administration Hospital.

ventricle, through the mitral orifice and out the atrial appendage (Fig 1a) The prosthesis is attached and drawn retrograde into position below the incompetent mitral leaflet The effect on the jet of insufficiency is readily palpable A similar technique (Fig 1b) can be used in the aortic area utilizing a side arm sutured to the aorta

RESULTS

In the process of placing the mitral prosthesis a few animals were found to have spontaneous organic mitral insufficiency The jet of insufficiency was stopped in these animals as well as in animals with induced insufficiency (Fig 1c) In the aortic area no spontaneous insufficiencies have been found and many methods have been tried to create an aortic insufficiency that is anatomically comparable to that occurring in humans To date no method has been successful although hemodynamic aortic insufficiency is easy to create by many methods It can be demonstrated that there is no obstruction to the outflow tract

The fate of the prosthesis placed in the circulation has been closely followed A few minutes after insertion the interstices of the nylon become filled with fibrin In about 18 hr tissue begins to grow onto the shaft of the prosthesis from the endocardium and the frame is completely covered in 12 to 14 days with a smooth glistening endothelial like substance (Fig 2a) Microscopically it is noted that the nylon is invaded by fibrous tissue which is more dense at the surface (Fig 2b) After maturation there appears to be little or no alteration in the tissue with time The metallic frame can be seen fluoroscopically and motion up to 2½ yr is noted In the living animals there has been no sign of emboli and in animals sacrificed up to 22 mo there has been no clot on the prosthesis itself or on adjacent endocardium The reticulocyte counts have not been altered and Lee White clotting times have been normal

DISCUSSION

The valvulogenic properties of this nylon covered Elgiloy frame are demonstrated The prosthesis stops insufficiency in the acute preparation in animals The internal resistance of the spring gives shape to the flap and also prevents the valve from becoming stuck to the wall of the heart or aorta Unipoint fixation not only makes the insertion easier but minimizes unnatural eddies which could beget fibrin deposits which could embolize

The technique of insertion in the mitral area has proven quite simple and safe The aortic approach is a bit more difficult because of the friability of the aorta and the higher pressure in the aorta

Studies have been carried out on survivals which indicate that a similar procedure is effective in the area of the aortic valve and gives promise of a method of treatment for aortic stenosis and insufficiency

SUMMARY

1 Criteria for valvular prosthesis have been formulated
2 A spring valve is described
3 Efficiency and valvulogenic properties are discussed
4 Application to the treatment of aortic stenosis is considered

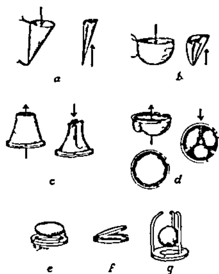



Fig 2 Types of subcoronary aortic valves employed a) Dixie Cup valve b) Rounded Dixie Cup valve in plastic ring seat c) Windsock valve d) Tricuspid valve e) Neumann Bailey Polywog valve f) Flap valve g) Ball valve

more places and the apex fixed to the antero medial aspect of the left ventricle immediately below the first branch of the anterior descending branch of the left coronary artery In such a location the prosthetic valve does not interfere with the aortic leaf of the mitral valve During systole the cone collapses allowing unimpeded blood flow into the aorta During diastole the valve pops open the cone fits snugly into the annulus and regurgitation is prevented

A total of 21 such valves have been tested—13 with a single layer of nylon and 8 of fused nylon and polythene The valve was variously fixed to the annulus with a single suture (4 animals) with 2 opposed sutures (3 animals) and with a running stitch one half way around the annulus (14 animals)

The longest survival with the use of such conical valves as a replacement after total excision of the aortic leaflets has been 1 hr These are easily seated theoretically will be replaced by the host tissue and minimally obstruct ventricular outflow but there is an almost insuperable degree of fatigue on the plastic cloth and thrombosis occurs in the acute angle of the apex when emptying is not complete

*Type 2—Rounded Dixie Cup Valve (Fig 2b)* In order to avoid thrombosis in the apex of the conical valve a cloth valve of similar design but with a rounded bottom has been employed in 2 instances Such valves shaped much like an aortic leaflet do not thrombose but are more difficult to seat and there have been no long term survivals

Fused nylon polythene valves have been employed in both instances but this makes the valves more bulky and complicates the problem of stress and tissue fatigue

*Type 3—Wind Sock Valve (Fig 2c)* A nylon cloth valve shaped like a wind sock with the base sutured to the annulus and the free end extending into the aorta above the coronary ostia has been employed in one animal In cardiac diastole the valve collapses and the outflow tract is obliterated The unsatisfactory features of this valve were its tendency to turn inside out under severe pressure and the partial obliteration of the coronary ostia when the valve collapses during cardiac diastole

*Type 4—Tricuspid Valve (Fig 2d)* Insertion of a tricuspid nylon cloth

and in 13 animals chemically induced cardiac arrest using potassium citrate[6,7] was employed

In the other 33 animals the ascending aorta was clamped and the remainder of the body perfused with oxygenated blood utilizing the Lillihei De Wahl technique[8] and an oxygenator of our own design

In order to provide coronary perfusion while the root of the aorta and the coronary ostia are excluded from the circulation a #18 polythene catheter attached to a side arm of the pump oxygenator has been inserted into the left coronary ostium (Fig 1) A small bulbous tip was fashioned on the end of the catheter to facilitate its insertion into the proximal left coronary artery and to avoid puncturing this friable vessel The small catheter does not obstruct the surgical field and provides adequate coronary perfusion during the period of open left cardiotomy

In general it would seem that chemically induced cardiac diastole combined with perfusion of the aorta above the occluding clamp is the optimal arrangement If coronary perfusion is indeed necessary[9] it can be provided by a separate perfusion catheter

Air embolism has not proven to be a serious problem With the coronary ostia exposed through the aortotomy incision there is no evidence of blood flow in the coronary vessels and consequently air is not introduced into the coronary system At the time of aortotomy closure the ventricle is filled by blood from the coronary catheter as it is withdrawn from the ostium and the occasional small air bubbles found in the coronary vessels pass through the coronaries as soon as the infusion pressure of the pump is directed into the coronary vessel after release of the aortic clamp

**Valve Design** Nylon mesh has been utilized in the construction of the cloth valves in this study In 8 animals a layer of polythene film has been fused to the mesh[10] in order to make it impervious to blood and therefore to make the valve immediately competent All solid valves and valve rings have been made of polyester resin plastic No attempt has been made to develop an ideal plastic for these valves This problem must await their more successful design

Immediately prior to the insertion of the various prosthetic valves the aortic valves were routinely excised with scissors under direct vision

*Type 1—Dixie Cup Valve (Fig 2a)* This valve is made of nylon cloth cut in the shape of a cone with the open end attached to the annulus in one or

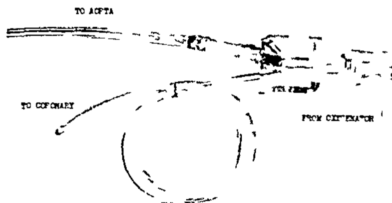



Fig 1 Bulb tip side arm catheter from pump oxygenator used for perfusion of left coronary artery

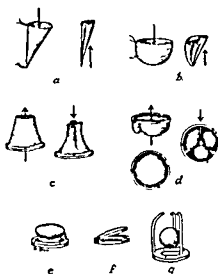



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*Type 4—Tricuspid Valve (Fig 2d)* Insertion of a tricuspid nylon cloth

valve similar in design to the normal aortic valve has been attempted in 14 animals The 3 round bottom cusps are fused to each other and sutured circumferentially to the annulus The cusps have been seated in a plastic ring sutured to the annulus in 4 animals The cusps of these valves must in some way be fixed below the annulus yet impingement on either the aortic leaf of the mitral valve or on the interventricular septum must be avoided As utilized in this study the cusps materially encroached upon the aortic outflow tract during systole

*Type 5—Neumann Bailey Polywog Valve (Fig 2e)* A modification of the Neumann Bailey flap ball valve[3] has been employed in 2 animals with a plastic seating ring sutured to the annulus These valves produce appreciable aortic stenosis, and their seating on the annulus also interferes with blood flow into the coronary ostia

*Type 6—Flap Valve (Fig 2f)* A circular lucite disc of a diameter slightly less than that of the annulus and hinged to a plastic ring seated in the annulus was inserted with but temporary success in 6 animals This type of valve is easily placed and produces a minimum of aortic stenosis

*Type 7—Ball Valves (Fig 2g)* Ball valves of various design have been employed unsuccessfully in 4 cases The success of the Hufnagel ball valve[11] in the descending aorta suggests its use in the subcoronary region but there are difficulties in such an adaptation, for unless provision is made for enlarging the diameter of the aorta immediately above the annulus where the ball is seated there will be serious aortic stenosis

## DISCUSSION

A successful subcoronary aortic valve has not as yet been achieved The surgical approach to the area is feasible Adequate perfusion both of the myocardium and the remainder of the body can now be attained for the time required for excision of the aortic valve and its prosthetic replacement The main difficulty therefore lies in the proper design of the valve If it is to be collapsible it should eventually be replaced by host tissue of an equally flexible nature in order to obviate the ultimate insuperable problem of finding a plastic that will not disintegrate under such conditions If it is to be a ball valve the cross sectional area of the outflow tract immediately distal to the seated valve must be sufficiently large to avoid stenosis

Valve placement is most easily accomplished by attaching the valve to a plastic ring which in turn is sutured to the aortic annulus Since the annulus moves and changes its shape during the cardiac cycle such a plastic ring should also be flexible An incompletely circular ring best provides for such expansion and contraction

## SUMMARY

1 Although a successful prosthetic subcoronary aortic valve has not yet been achieved our experience in the design and placement of 7 types of such valves in a series of 53 dogs is reported

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# THE SURGICAL PRODUCTION OF CHRONIC PROGRESSIVE MITRAL INSUFFICIENCY IN DOGS*

Sam J Kuykendall F Henry Ellis Jr John H Grindlay and Earl H Wood

A practical method of creating mitral insufficiency in animals with subsequent survival is desirable in order that hemodynamic changes associated with the condition may be studied and various surgical techniques for its correction tested

Many investigators have produced acute mitral regurgitation experimentally by inserting instruments through the left atrial appendage or the left ventricular wall in order to cut the cusps of the valves sever chordae tendineae or excise portions of the cusps Usually these procedures produce such extensive valvular damage that the degree of regurgitation is difficult to control and prolonged survival of the animals has been rare

Haller and Morrow[1] have been able to produce a more controlled degree of mitral insufficiency with prolonged survival in 4 of 15 dogs by selective cutting of the main supporting chordae to the cusps through a closed ventricular approach Crawshaw Vetten and Wilson produced regurgitation by suturing the chordae of the posterior cusp back to the ventricular wall and obtained prolonged survival in 6 of 11 dogs The chordae were selected

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* Mayo Clinic and Mayo Foundation Rochester Minnesota The Mayo Foundation is a part of the Graduate School of the University of Minnesota

valve similar in design to the normal aortic valve has been attempted in 11 animals. The 3 round bottom cusps are fused to each other and sutured circumferentially to the annulus. The cusps have been seated in a plastic ring sutured to the annulus in 4 animals. The cusps of these valves must in some way be fixed below the annulus yet impingement on either the aortic leaf of the mitral valve or on the interventricular septum must be avoided. As utilized in this study, the cusps materially encroached upon the aortic outflow tract during systole.

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## DISCUSSION

A successful subcoronary aortic valve has not as yet been achieved. The surgical approach to the area is feasible. Adequate perfusion both of the myocardium and the remainder of the body can now be attained for the time required for excision of the aortic valve and its prosthetic replacement. The main difficulty therefore lies in the proper design of the valve. If it is to be collapsible it should eventually be replaced by host tissue of an equally flexible nature in order to obviate the ultimate insuperable problem of finding a plastic that will not disintegrate under such conditions. If it is to be a ball valve the cross sectional area of the outflow tract immediately distal to the seated valve must be sufficiently large to avoid stenosis.

Valve placement is most easily accomplished by attaching the valve to a plastic ring which in turn is sutured to the aortic annulus. Since the annulus moves and changes its shape during the cardiac cycle, such a plastic ring should also be flexible. An incompletely circular ring best provides for such expansion and contraction.


## SUMMARY

1. Although a successful prosthetic subcoronary aortic valve has not yet been achieved, our experience in the design and placement of 7 types of such valves in a series of 53 dogs is reported.


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RESULTS

This report concerns a consecutive series of 29 dogs in which mitral insufficiency was created by this technique There was one operative death due to cardiac arrest Six deaths were due to cardiac failure and acute pulmonary edema These occurred from 7 to 51 days after operation The over all prolonged survival rate for this series was approximately 75 per cent The procedure has been performed subsequently in a number of dogs with an even higher survival rate

The degree of regurgitation had been graded at the time of creation in the 22 survivors as mild in 4 moderate in 12 and marked in 6 Of the 4 dogs with mild mitral insufficiency 3 presented murmurs postoperatively and only 1 of these showed significant dyspnea or gave roentgenologic evidence of cardiac enlargement At re exploration 76 to 120 days after creation the degree of regurgitation was increased in 1 unchanged in 1 and decreased in 2 In none of these animals with mild insufficiency did any dilatation of the mitral ring develop

In contrast all of the 12 animals with moderate regurgitation presented systolic murmurs and exertional dyspnea and two-thirds showed significant limitation of activity or gave roentgenologic evidence of cardiac enlargement

Furthermore all of the 6 animals in which regurgitation at the time of its creation was of marked degree presented systolic murmurs immediately post operatively and the murmurs persisted Also all of these dogs were dyspneic on exertion were limited in their activity and gave roentgenologic evidence of cardiac enlargement

The 18 dogs with moderate or marked regurgitation were explored surgically 7 to 27 wks after creation of the mitral insufficiency The amount of regurgitation as determined by palpation was unchanged in 3 of the dogs and was increased in intensity in 15 (83 per cent) of the 18 Intra atrial palpation gave the gross impression of definite secondary dilatation of the mitral ring in 17 (94 per cent) of the 18 dogs In all 18 dogs the left atrium and left ventricle were enlarged and in those dogs in which regurgitation had been greatest the right ventricle was enlarged as well

In 11 dogs the intensity of the systolic murmur had increased before exploration In all of these 11 the mitral rings were found to be dilated and the degree of insufficiency was found to be greater than had been created originally The observation suggests that in this series of dogs the increase in the intensity of the murmur was indicative of the development of secondary dilatation of the annulus The impression obtained by palpation that such dilatation occurred in 17 of the 18 dogs demonstrates the progressive pathologic and physiologic changes that develop in dogs with prolonged mitral insufficiency

Simultaneous left atrial and left ventricular pressures were recorded for some of the dogs The changes in contour and pressure closely paralleled those seen in human beings with mitral insufficiency The left atrial mean pressure consistently was increased the average being 11 3 mm Hg as compared with the preregurgitation average of 3 0 mm There was prominent elevation of the V wave

Indicator-dilution studies also were performed in some instances The dye

and the suture carrier guided by a finger inserted through the left atrial appendage

METHOD

Anatomic study of the region of the mitral valve in fresh specimens obtained at necropsy suggested the possibility of creating mitral regurgitation by hooking the chordae of the mural (posterolateral) cusp and tacking them to the left ventricular wall A direct left ventricular approach was used leaving the atrial appendage intact for later use in gaining access to the valve region for applying corrective procedures A small blunt nerve hook was inserted through a small epicardial incision at a point 1 5 cm anterior to the left marginal coronary vessels and 1 cm below the circumflex coronary vessels (Fig 1) Use of this site avoided injury to the larger coronary vessels and damage to the papillary muscles and the leaflets Chordae which supported the lateral one third to one half of the mural (posterolateral) cusp were engaged with the hook Then while steady traction on the instrument held the tendons against the endocardium a heavy cotton suture was passed through the ventricular wall and back encircling the ensnared chordae A modified aneurysm needle was used to pass the suture After careful withdrawal of the hook disengaging the chordae without severing them the ends of the suture were tied over a small square of compressed polyvinyl (Ivalon) sponge placed on the epicardium The extent of immediate ballooning of the atrial appendage and the intensity of the palpable thrill over the left atrium indicated the degree of regurgitation produced

This method has the advantage of producing shortening of the chordae and retraction of the mural cusp simulating the condition usually seen in human beings with rheumatic mitral insufficiency except that the resulting defect is in the region of the anterolateral commissure rather than that of the posteromedial commissure In the dogs that have been killed subsequently the ensnared chordae and a small portion of the retracted cusp have been found to be firmly adherent to the endocardial surface of the left ventricle

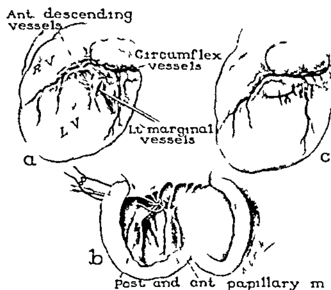



Fig 1 *a* The site of the incision the hook engaging chordae is within the left ventricle *b* The hook holding chordae of the posterolateral cusp against the endocardium during placement of the encircling suture *c* The position of the finished suture The polyvinyl sponge over which the suture is tied is not shown in the drawing

in order to study changes in the pulmonary artery This paper will present this study

### METHOD

Mitral stenosis was produced in 8 dogs by the previously described method[1] The dogs were anesthetized with intravenous pentobarbital sodium 2 wks before and from 2 wks to 13 mo after production of experimental mitral stenosis With the animal supine cardiac catheters were introduced into the pulmonary artery and vein Pulmonary artery pulmonary vein and intrathoracic pressures were recorded with standard resistance wire pressure transducers (Statham Strain Gauges) Zeros were arrived at by visualizing the tips of the catheters fluoroscopically and exposing the strain gauges to atmospheric pressure at that level Pressures were obtained in all 8 dogs just before sacrifice The circumference and the diameter of the mitral valve were measured in the dogs after sacrifice Since the orifices were circular the area was calculated from the circumference Using Raubner's constant in Meek's Formula 11 2 (wt in gm) to calculate surface area the valve area was then expressed per square meter of body surface area The lungs were sectioned and prepared for microscopic examination

### RESULTS

Four of the dogs sacrificed showed no appreciable narrowing of the mitral valve The remaining 4 dogs had a moderate to a marked degree of experimental mitral stenosis

The dogs with no mitral narrowing had an average pulmonary vein pressure of 7 5 mm Hg and a pulmonary artery pressure of 16 5 mm Hg In the 4 animals with marked narrowing of the mitral opening the pulmonary vein pressures averaged 16 5 mm Hg and the pulmonary artery pressure averaged 25 mm Hg (See Table 1) The lung sections were read by Dr John F Noble Pathologist at Ancker Hospital They were also seen by Dr Jesse Edwards of the Mayo Clinic Both of these men were shown the slides without any knowledge of the dog from which they came Each was able to distinguish changes in the small pulmonary arteries of the 4 dogs with the most mitral narrowing (See Table 2)

These changes consisted for the most part of medial thickening but with occasional vessels showing some intimal thickening

*Table 1 Average Pressures in Pulmonary Artery and Pulmonary Vein*

| DOG NUMBER | WEIGHT | VALVE SIZE cm²/m² | PULMONARY VEIN | PULMONARY ARTERY |
|---|---|---|---|---|
| 1 | 45# | 5 8 | 6 | 12 |
| 2 | 45# | 1 3 | 8 | 18 |
| 3 | 44# | 4 6 | 10 | 16 |
| 4 | 43# | 2.5 | 5 | 19 |
| 5 | 46# | 9 | 20 | 24 |
| 6 | 40# | .8 | 15 | 21 |
| 7 | 43# | 4 | 23 | 32 |
| 8 | 39# | .5 | 18 | 25 |

(Evans blue) was injected into the left ventricle and simultaneously recorded at the left atrium and the femoral artery The dye constantly appeared in large quantities in the left atrium before it appeared in the femoral artery

### SUMMARY AND COMMENT

A method is presented of producing mitral insufficiency in dogs which is fairly well controlled in degree and also compatible with prolonged survival The anatomic defects produced—shortening of chordae tendineae retraction of the cusp and secondary dilatation of the mitral ring—are similar to the pathologic findings in human beings with mitral insufficiency of rheumatic origin The hemodynamic changes and the clinical manifestations of murmurs dyspnea and cardiac enlargement are comparable to those of patients who are suffering from this disease This experimental preparation may be of value in the further investigation of hemodynamic changes and of methods of correcting mitral insufficiency

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# PULMONARY ARTERY CHANGES IN MITRAL STENOSIS*
## *An Experimental Study*

DONALD W HANNON ALLAN FERRIN, FRANCIS HADDY,
JOSEPH L SPRAFKA AND IVAN D BARONOFSKY

As more and more of the long term results of mitral commissurotomy are received it is obvious that the operation is of marked benefit in about 70 per cent of patients operated upon One of the obstacles to a successful result of this operation has been fixed organic changes that have occurred in the pulmonary artery as a result of prolonged pulmonary venous hypertension Adequate experimental studies of pulmonary artery changes have been hampered in the past by the lack of a good method for producing experimental mitral stenosis At the 1951 Forum meeting two of us[1] studied the problem extensively and reported an experimental method of producing mitral stenosis The method consisted of inversion of the left auricular appendage across the mitral ring above the valve Very marked narrowing of the mitral orifice was obtained Using this method the hemodynamics of mitral stenosis were studied[2 4 5] In addition a group of 8 dogs was allowed to live for 3 to 4 yr

*From the Department of Surgery University of Minnesota and the Surgical Research Laboratories of Ancker Hospital St Paul Minn

in order to study changes in the pulmonary artery This paper will present this study

METHOD

Mitral stenosis was produced in 8 dogs by the previously described method[1] The dogs were anesthetized with intravenous pentobarbital sodium 2 wks before and from 2 wks to 43 mo after production of experimental mitral stenosis With the animal supine cardiac catheters were introduced into the pulmonary artery and vein Pulmonary artery pulmonary vein and intrathoracic pressures were recorded with standard resistance wire pressure transducers (Statham Strain Gauges) Zeros were arrived at by visualizing the tips of the catheters fluoroscopically and exposing the strain gauges to atmospheric pressure at that level Pressures were obtained in all 8 dogs just before sacrifice The circumference and the diameter of the mitral valve were measured in the dogs after sacrifice Since the orifices were circular the area was calculated from the circumference Using Raubner s constant in Meek s Formula 11 2 (wt in gm) to calculate surface area the valve area was then expressed per square meter of body surface area The lungs were sectioned and prepared for microscopic examination

RESULTS

Four of the dogs sacrificed showed no appreciable narrowing of the mitral valve The remaining 4 dogs had a moderate to a marked degree of experimental mitral stenosis

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|---|---|---|---|---|
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| 3 | 44# | 4 6 | 10 | 16 |
| 4 | 43# | 2.5 | 5 | 19 |
| 5 | 46# | 9 | 20 | 24 |
| 6 | 40# | 8 | 15 | 21 |
| 7 | 43# | 4 | 23 | 32 |
| 8 | 39# | .5 | 18 | 25 |

*Table 2*

| DOG NUMBER | VALVE SIZE $cm^2/m^2$ | TIME STENOSIS PRESENT | THICKENING OF SMALL ARTERIES |
|---|---|---|---|
| 1 | 5 8 | 3 yrs 2 wks | 0 |
| 2 | 1.3 | 3 yrs 2 wks | 0 |
| 3 | 1 6 | 3 yrs 3 mo | 0 |
| 4 | 2 [illegible] | 3 yrs 1 mo | 0 |
| 5 | 9 | 3 yrs 1 mo | Present |
| 6 | 8 | 3 yrs 2 wks | Present |
| 7 | 4 | 3 yrs 7 mo | Present |
| 8 | .5 | 3 yrs 8 mo | Present |

We are deeply indebted to Dr John F Noble of Ancker Hospital and Dr Jesse Edwards of the Mayo Clinic for their help in examining these lung sections

There seemed to be a good correlation between mitral orifice size and the small artery changes No changes were noted where the valve size was over 1 cm in diameter

DISCUSSION

The changes noted in the small pulmonary arteries of the dogs with experimental mitral stenosis are moderate in amount but the fact that they occur is most important It would have been interesting to see how soon these changes would regress with reopening of the narrowed mitral area

It may be surprising to find that only moderate increase in pulmonary artery pressures was observed in dogs with arterial changes In a previous publication we have shown that pulmonary resistance is inversely related to the pulmonary vein pressure In all probability pressures in the pulmonary artery will rise to high levels not as a result of high pulmonary vein pressures but as a result of very advanced organic changes in the pulmonary artery In these animals the changes are present but haven t advanced to that stage Therefore little emphasis can be placed on pulmonary artery pressures as an indication of the presence or absence of small artery thickening The early development of these changes probably represents a very strong argument in favor of the early operation of mitral stenosis Pulmonary hypertension once it is fully developed may represent progression of the arterial changes to an irreversible state Certainly the changes which are noted in our experimental group could possibly be expected to revert as opposed to the changes seen with well established pulmonary hypertension Even if they remained as is it can be stated that the work load of the right ventricle would not be increased any further if operation were successfully accomplished A word might be interjected at this time regarding the procedure of producing the stenosis The ease with which narrowing of the mitral orifice can be accomplished by this method suggests the possibility of using a variation of the technique in the treatment of mitral insufficiency

CONCLUSIONS

1 Effective chronic narrowing of the mitral ring can be accomplished easily by the method previously described

2 Dogs with moderate to marked experimental narrowing of the mitral area developed small artery changes consisting of medial thickening and occasional intimal thickening after 37 to 44 months

3 The small artery changes developed in the absence of any marked elevation of pulmonary artery pressures

4 It would not seem advisable to wait for elevation of pulmonary artery pressures as an indication for surgery in mitral stenosis

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## MEASUREMENT OF THE ELECTRICAL IMPEDANCE OF THE HUMAN HEART DURING DEFIBRILLATION*

CHARLES K KIRBY JOSEPH ENGELBERG AND JULIAN JOHNSON

Several years ago we observed that much stronger shocks were apparently required for successful defibrillation of the ventricles of patients with large hearts than of those with hearts of approximately normal size We then studied the problem of optimum voltage in dogs and found that single shocks of 270 volts were considerably more effective than single shocks of 110 volts in abolishing ventricular fibrillation of 4 min duration [1] Shocks up to 270 volts caused no grossly visible burns or other ill effects when the duration did not exceed 0 10 sec On the basis of this evidence a timing device and high voltage transformer were incorporated in our operating room defibrillator We adopted the policy of administering a high voltage shock of short duration in patients with large hearts rather than beginning with low voltage shocks and working upward as had formerly been our practice The single high voltage shocks were usually successful

The apparent need for stronger shocks for larger hearts raised the question as to whether large hearts had greater electrical impedance or whether other factors associated with cardiac enlargement made defibrillation more

*From the Harrison Department of Surgical Research Schools of Medicine University of Pennsylvania Philadelphia Supported by a grant from the York County (Pennsylvania) Heart Association

difficult No information concerning the impedance of the heart in living humans could be found in the literature

We therefore designed a defibrillator to measure the cardiac impedance during the application of shocks in patients with ventricular fibrillation This defibrillator has been used in the operating room for nearly two years

METHOD

The circuit diagram of the defibrillator used in these studies is shown in Figure 1 The electrodes were made of stainless steel and were 6 4 cm in diameter They were applied directly to opposite sides of the heart The handle of each electrode was insulated but the back was not Moderate firm pressure was used to reduce the distance between the electrodes An attempt was made to keep the amount of electrode compression relatively uniform and we believe that this was possible since the electrodes were handled by the same operators in nearly every patient Single 0 10 sec. shocks of from 120 to 230 volts (60 cycle per sec) were used in most instances and each single shock usually stopped fibrillation Repetition of the shock was often necessary because fibrillation recurred Serial shocks were sometimes needed especially after single shocks had been used repeatedly for 30 min or more

The defibrillation current was recorded on a Sanborn Model 127 Recorder The apparatus was calibrated with known impedances and currents The current passing through the heart and the heart impedance, as reflected in the height of the deflection in millimeters was determined from a calibration chart

The recordings made on 28 patients were available for analysis In 22 of the patients the operative procedure involved the heart or great vessels

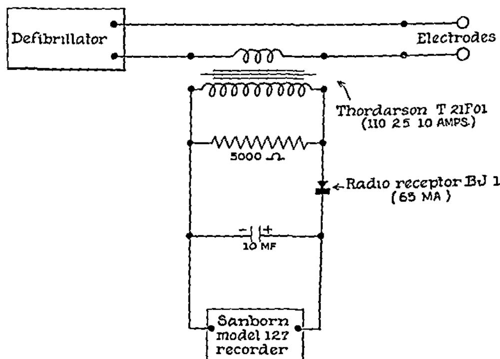



Fig 1 Circuit used with the defibrillator to measure and record the current passing through the heart Constructed for us by American Electronics Laboratories Inc 641 Arch Street Philadelphia Penna

Four patients had a pulmonary resection 1 a cholecystectomy, and 1 a craniotomy for brain tumor

### RESULTS

Of the 28 patients 4 recovered completely and were discharged from the hospital Seven lived from 1 to 36 hr postoperatively and the remaining 17 did not survive the operative procedure Ventricular fibrillation was abolished in every patient In many patients it recurred often repeatedly, but death was due to inability to establish an effective heart beat rather than to intractable fibrillation

The electrical impedance of the hearts of the 28 patients ranged from 20 5 to 49 5 ohms with a median of 35 7 ohms * The current ranged from 2 7 to 8 8 amperes with a median of 5 7 amperes The accuracy of measurement of heart impedance was estimated to be plus or minus 10 per cent and of current plus or minus 5 per cent The heart weight was determined at autopsy in 17 patients The weight varied from 310 to 900 gm with a median of 450 gm There appeared to be little evidence of correlation between heart weight and impedance the correlation coefficient being plus 0 1

### DISCUSSION

Mackay Mooslin and Leeds[7] found that the average resistance of the living dog heart was about 50 ohms Beattie Keshishian Ames and Blades[3] confirmed this observation and also studied dead dog hearts which had an average resistance of 97 ohms and dead human hearts which had an average resistance of 93 ohms

In this study we have found that the impedance of large human hearts was not greater and actually appeared to be less than that of live dog hearts which are considerably smaller There appears to be little if any correlation between heart size and impedance This is strikingly illustrated by the fact that the impedance of the largest heart in this series (900 gm aortic stenosis) was only 26 2 ohms which was well below the average for the entire series of 35 7 ohms

Our previous suggestion[1] that a transformer be used to produce voltages higher than the usual house current (110–115 volts) for defibrillation of large hearts received support in this study In many instances shocks of 230 volts were successful after shocks of lower voltage had failed

Since there appears to be no significant correlation between electrical impedance and heart size the need for higher voltages for larger hearts is apparently due to factors other than increased resistance It seems likely that such factors include the disease process itself as well as hemodynamic and metabolic abnormalities which are the cause or the result of cardiac enlargement

### CONCLUSIONS

1 The electrical impedance of and the current passing through the human heart was measured during the application of electric shocks of 120 to 230 volts in 28 patients in ventricular fibrillation

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* In most patients receiving multiple shocks the variation of the heart impedance about the patient's mean impedance was ± 15 per cent (occasionally as high as ± 30 per cent) This was probably due to variations in electrode compression

2 The impedance ranged from 20 5 to 19 5 ohms with a median of 35 7 ohms It appeared to be lower than that of the living dog heart

3 The need for high voltage shocks in defibrillating large human hearts appears to be due to factors other than an increase in electrical impedance

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# THE EXPERIMENTAL PRODUCTION OF ARRHYTHMIAS BY SELECTIVE DESTRUCTION OF THE CARDIAC CONDUCTION SYSTEM*

ROBERT W HARRISON PETER V MOULDER JR PAUL V HARPER AND WILLIAM E ADAMS

Present day cardiac surgery has given little attention to the feasibility of correction of rhythm disturbances and conduction system defects Experimental subjects for the evaluation of medical and surgical therapy of arrhythmias were sought in these experiments by the selective destruction of segments of the conduction system and observation of the rhythm produced

Physiologic studies of the function of the conduction system by early investigators were frequently performed on perfused heart preparations or as acute experiments on the heart *in situ* In the following experiments well defined conduction system defects were produced by radiation from beta ray point sources In contrast to the earlier studies these were gradual in production and chronic in nature the animals being observed during the period of development of the lesion and for long periods of time thereafter A histochemical technique was utilized for accurate demonstration and microscopic delineation of the limits of the conduction system and for evaluation of the extent of the lesions produced

METHOD

Operations were performed on 18 mongrel dogs The exact details of the operative technique vary depending upon the portion of the conduction system attacked

*From the Department of Surgery and the Argonne Cancer Research Hospital University of Chicago Clinics Chicago Illinois This study aided by grants from the Oscar Mayer Foundation and the Douglas Smith Foundation for Medical Research of the University of Chicago

The point source radiation was provided by yttrium oxide pellets containing 0 5 to 1 5 mc of Y[90] activity The cylindrical pellets were 0 5 mm in diameter and 1 to 1 5 mm in length Details for the preparation of yttrium oxide pellets have been described[1] The radioactive pellets were handled by insertion in lead shielded 17 gauge spinal needles The tip of the needle was inserted into the tissue to the desired location and the pellet then ejected by means of the obturator of the needle The radioactive yttrium pellets emit beta radiation which has a half life of 62 hr and a maximum range in tissue of approximately 1 0 cm The areas of necrosis produced by these beta ray point sources are sharply demarcated approximately 8 mm in diameter

Pockets of various material (free pericardial graft free aortic wall homo graft and thin polyethylene sheeting) were fashioned to cover generously the area of the right atrium which contained the sino atrial node The pockets were sutured to the surface of the atrium on the posterior wall at the cavo atrial junction and along the sulcus terminalis with #5 0 silk Radioactive pellets were inserted in the pockets in contact with the atrial wall spaced at 5 to 6 mm intervals Five to 8 pellets were used depending upon the size of the animal and the activity of the pellets

Hypothermia (24 to 26°C ) and complete interruption of the circulation with the employment of acetylcholine to render the heart essentially asys tolic were utilized to permit performance of a right atriotomy and visuali zation of the area of the inter atrial septum containing the atrio ventricular node[2] In the area between the ostium of the coronary sinus and the septum membranaceum 4 to 5 pellets were implanted subendocardially spaced approximately 5 mm apart

Preoperative and serial postoperative standard and augmented unipolar limb lead electrocardiograms were obtained on all animals

The histochemical technique utilized to demonstrate and study the loca tion of the specialized muscle tissues that make up the cardiac conduction system depend upon the demonstration of cholinesterase activity[3] Routine histologic studies were also performed Histochemical studies were per formed on a series of normal hearts as well as those hearts in which conduc tion system lesions had been produced to determine the location and extent of the lesions

## RESULTS

The normal conduction system in the dog s heart can be demonstrated enzymatically The enzyme is localized in the myofibrils of the specialized cardiac muscle fibers The boundaries of the conduction system have a sharp outline and no transition is seen between the specialized muscle fibers and the cardiac muscle cells

The site of the enzymatic reaction representing the sino atrial node was found to be in the crista terminalis The limits of the sino atrial node were sharply demonstrated by a positive zone which occupied a portion of the posterior wall of the right atrium and extended inferiorly along the sulcus terminalis to a point near the junction of the inferior vena cava with the right atrium

The atrio ventricular node occupies a position in the lower portion of the interatrial septum between the ostium of the coronary sinus and the septum membranaceum

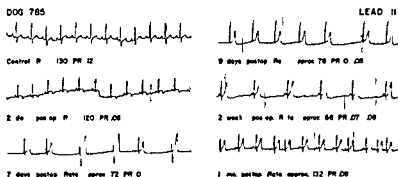



Fig. 1. Electrocardiographic tracings following the implantation of five 0.5 mc $Y^{90}$ pellets in the region of the sino-atrial node.

2 days postop.—Essentially normal EKG except for reduction of PR interval from .12 to .08 sec.

7 days postop.—Marked arrhythmia. P waves absent. Intermittent escape beats.

9 days postop.—Marked arrhythmia. P waves seen only occasionally. QRS complex of normal configuration.

2 weeks postop.—P wave constantly present with P-R interval reduced to .07 to .08 sec.

1 mo. postop.—Permanent rhythm established. Note decreased amplitude of P wave and decreased P-R interval.

**Sino-atrial node.** Total ablation of the sino-atrial node as evidenced by the absence of enzymatic activity histochemically results in only minimal permanent rhythm disturbances and electrocardiographic alterations. In the early postoperative period, rhythm disturbances are quite variable and unpredictable. A nearly constant finding is the diminution in amplitude or disappearance of the P wave which has been observed to be markedly reduced in potential within 24 hr. after the implantation of the pellets and has been noted to be absent as early as 48 hr. postoperatively. Not uncommonly there is a marked reduction in cardiac rate during this early period. Frequently in the early period there is an absence of the P wave which is associated with a normal appearing QRS complex and a nonphasic arrhythmia, in other words, a rhythm more "sinus" than "nodal." In those cases where the P wave remains visible, the P-R intervals show a definite shortening from a control value of .09 to .12 sec. to .06 to .08 sec. During the first 2 to 3 wks. following the implantation of the radioactive material there may be observed frequently intermittent periods of sino-auricular block or auricular standstill with associated beats of "nodal" origin or so-called "escape beats." In 2 to 3 wks. the permanent pattern is established with a rate approximating that of the control but with decreased amplitude of the P wave and a shortened P-R interval. Figure 1 illustrates these electrocardiographic changes.

**Atrio-ventricular node.** Radioactive $Y^{90}$ pellets implanted in the region of the atrio-ventricular node do not constantly produce an atrio-ventricular heart block. In the animals in which heart block developed the rhythm disturbance was first manifested by a prolongation of the atrio-ventricular conduction time represented by a prolonged P-R interval. Often this P-R interval would be variable in duration before becoming constantly prolonged and later (1 wk.) complete heart block would develop with its

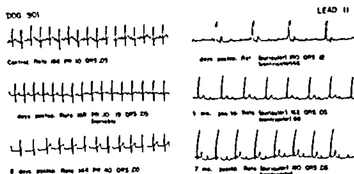



Fig 2 Electrocardiographic tracings following the implantation of four 1 mc $Y^{90}$ pellets in the region of the atrio ventricular node

4 days postop The P R interval is variable ranging from 10 to 19 sec Signifying a fluctuating degree of heartblock

6 days postop A constant first degree heart block with a P R interval of 40 sec Due to the prolonged P R interval the P wave is frequently located within the T wave of the preceding beat

7 days postop Complete heart block

1 mo postop Complete heart block

7 mo postop Complete heart block

typical atrio ventricular dissociation Figure 2 shows excerpts of serial electrocardiographic tracings of one animal in which a permanent heart block was produced Complete heart block has been observed to be permanent in those cases in which it developed

## DISCUSSION

Rhythm disturbances observed in the early period during progressive destruction of the sino atrial node i e sino atrial block auricular standstill and frequent escape beats are similar to the changes one would expect with removal of the pacemaker of the heart Other workers basing their conclusions on experiments in which the destruction or functional depression of the sino atrial node has been relatively sudden felt that the resulting rhythm arose from the coronary sinus or ventricular portion of the atrio-ventricular node

However the true site of impulse formation in these hearts is not readily apparent Due to the positive character of the P wave it must be concluded that the site of impulse formation in these hearts is located above the coronary sinus and the direction of spread of the impulse over the right atrium is normal Nevertheless the constant decrease in P R interval suggests the location of the site of origin close to the atrio ventricular node

It is felt that the slow destructive process used in these experiments simulates changes occurring clinically in most disease processes affecting the sino atrial node other than embolism or sudden thrombosis of the nutrient vessels The difficulty with which sino atrial block and auricular standstill are produced by gradual ablation of the sino atrial node corresponds with the rare clinical observation of such arrhythmias in conditions other than those associated with acute drug toxicity

The inconstancy in our ability to produce heart block by implantation of

the pellets in the region of the atrio ventricular node is more than likely dependent upon at least 2 factors First, as a result of auxomeric phenomena it is necessary to affect every fiber of the atrio ventricular system to produce a conduction disturbance Secondly although it is possible to place the pellets quite accurately, slight variations in the location of the atrio ventricular node would result in incomplete destruction and consequently no impairment of conduction

### CONCLUSIONS

1 Local beta radiation as produced by small radioactive yttrium[90] oxide pellets provides a means of gradually creating well localized defects in the conduction system of the heart

2 Gradual and complete ablation of the sino atrial node results in transient rhythm disturbances, i e auricular standstill, sino atrial block, and nodal rhythms but almost invariably the permanent rhythm is characterized by an upright P wave of decreased potential and a shortened P R interval

The site or origin of the cardiac impulse following ablation of the sino atrial node is not known

3 Permanent complete atrio ventricular heart block may be produced by application of local beta radiation in the region of the atrio ventricular node

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# EXPERIMENTAL CLOSURE OF RUPTURED SINUS OF VALSALVA ANEURYSMS*

R ROBINSON BAKER HANS ERIK HANSON AND ANDREW G MORROW

Rupture of a congenital aneurysm of the aortic sinus of Valsalva results in a fistulous communication between the base of the aorta and the right heart The usual sequelae are progressive cardiac failure and death A method for the closure of such fistulae was suggested during aortography in a patient with this lesion A catheter was easily passed from the aorta through the fistula and into the right atrium The course of the catheter suggested the experimental method described

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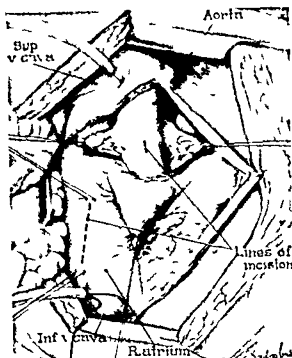



Fig 1 Operative exposure prior to inflow occlusion The sites of aortic and atrial incisions are indicated

## METHOD

The prostheses shaped like golf tees were made from compressed poly vinyl (Ivalon) sponge The diameter of the flattened head was 4 to 5 mm and that of the shaft 2 to 3 mm They were sterilized by boiling

Dogs weighing 15 to 20 kg were anesthetized with intravenous pentothal and cooled by immersion to a final rectal temperature of 33 C The right chest was entered through the fourth intercostal space and traction ligatures placed about the superior and inferior venae cavae The azygos vein was ligated and the pericardium opened anterior to the phrenic nerve The right atrial appendage was retracted inferiorly, exposing the root of the aorta The adventitia of the aorta was excised and retracted to each side by means of silk sutures Stay sutures were then placed in the body of the right atrium

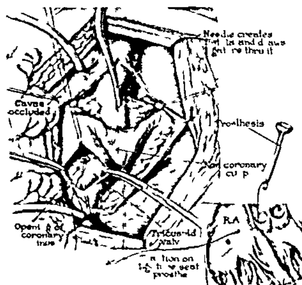



Fig 2 The fistula is created between the aorta and right atrium with a modified Reverdin needle A heavy ligature is drawn through it and the prosthesis introduced through the aortic incision

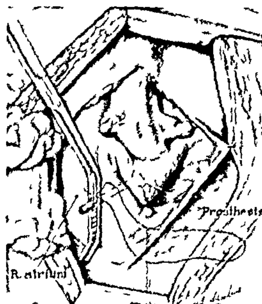



Fig. 3. The aortic and atrial incisions are closed; the tail of the prosthesis is incorporated into the atrial suture line.

Incisions were made in the aorta and right atrium following the application of partially occluding clamps (Fig. 1).

In 10 animals a fistula was created between the non-coronary aortic sinus and right atrium. Following inflow occlusion, the right atrial clamp was removed and the right index finger placed just superior and medial to the opening of the coronary sinus. A modified Reverdin needle was then introduced through the aortic incision and advanced into the non-coronary sinus until the tip was palpated by the finger within the right atrium. A tract was created by advancing the needle through the aortic wall and out into the atrium (Fig. 2). A heavy ligature was threaded into the eye of the needle and the needle withdrawn from the aorta. The ligature then passed from the aorta through the newly created tract and into the atrium. The shaft of the prosthesis was tied to this ligature and the prosthesis pulled into the aorta so that its proximal flattened end lay behind the aortic leaflet. The shaft occluded the tract and was brought out through the atrial incision. Partially occluding clamps were then reapplied to the aortic and atrial incisions and circulation re-established. These incisions were closed with continuous over-and-over sutures, the tail of the prosthesis being incorporated into the atrial suture line.

In 11 dogs, a tract was created between the right coronary sinus and right ventricle. In these dogs, the index finger was placed under the septal leaflet of the tricuspid valve. The Reverdin needle was introduced into the right coronary sinus and advanced into the right ventricle and then into the right atrium at a point just medial to the septal leaflet of the tricuspid valve. The prosthesis was introduced so that its head lay behind the right coronary leaflet and its shaft passed from the ventricle across the septal leaflet of the tricuspid valve. The tail of the prosthesis was again incorporated into the atrial suture line (Fig. 4).

There were three operative deaths in the 21 animals, 1 each from anesthetic overdosage, distemper, and a technical error. Aortic and left ventricular catheterizations were performed 1 wk. postoperatively. The animals were sacrificed at 1 to 4 wk. intervals following the operation.

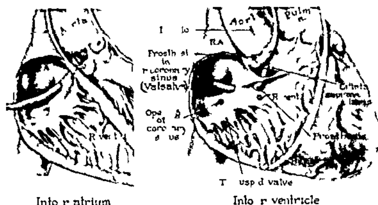



Fig 4 Final positions of the prosthesis in fistulae created between the non coronary sinus and right atrium and right coronary sinus and right ventricle

RESULTS AND DISCUSSION

There were no manifestations of aortic stenosis or insufficiency in any animals The aortic and left ventricular catheterizations showed no systolic gradients across the aortic valve and the arterial pressure pulses were normal At autopsy the aortic valve leaflets were undamaged The aortic and atrial portions of the prostheses were covered with smooth endothelium

Lillehei[1] and Bahnson[2] have recently closed cardio aortic fistulae by suture from the right side during total cardiac bypass Edwards[3] has pointed out that the essential pathologic feature of a congenital aneurysm of the sinus of Valsalva is a lack of continuity between the aortic media and the annulus of the aortic valve In the method described this continuity is restored by bridging the defect with a prosthesis introduced from the aortic side

The operation has recently been successfully performed in a 28 year old man in whom an aneurysm of the right coronary sinus had ruptured into the right atrium This case will be subsequently reported in detail

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# FURTHER STUDIES IN INDUCED CARDIAC ARREST USING THE AGENT ACETYLCHOLINE*

CHARLES K. SERGEANT, THOMAS GEOGHEGAN AND CONRAD R. LAM

The advantages of carrying out open heart operations on a motionless heart are obvious. In a completely bloodless and quiet field these procedures are greatly facilitated. The relatively successful use of potassium chloride as a cardioplegic agent was reported by us in 1955.[1] However, resuscitation was not invariably successful and was usually complicated by ventricular fibrillation. Therefore in November 1955 we decided to try the drug acetylcholine although we were aware that Bjork[2] had not been successful with it in at least one experiment.

The ability of acetylcholine to stop the heart was easily demonstrated in a series of acute experiments. Under endotracheal anesthesia with ether thoracotomy was carried out on 20 dogs. The *pericardium was opened*, the venae cavae occluded by snares, the ascending aorta occluded and 10 mg./kg. of acetylcholine (Acecholine, Anglo French Labs.) was injected into the proximal aorta where presumably most of it entered the coronary arteries. The commercial preparation was diluted 1:10 with saline solution. Prompt and complete cardiac arrest was produced (Fig. 1). Resuscitative measures were begun after 5 to 10 min. of arrest, the essential step being the perfusion of the coronary arteries with blood to wash out the cardioplegic drug. In 8 animals the perfusion was accomplished by forcing blood from a reservoir through a tube inserted through a subclavian artery, and in 12 animals ordinary cardiac massage was used. Following a total of 31 arrests, resuscitation was attained in all instances.

We then proceeded to use this method of elective cardiac arrest on animals in which the brain was protected by general hypothermia. Two types of intracardiac operations were carried out. In 20 animals partial aortic stenosis was produced by suturing 1 or 2 commissures, and in 4 animals ventricular septal defects were made and repaired. Cardiac rhythm was restored in each instance by massage. Twenty two of these animals survived; 2 died on the third postoperative day of hemorrhage from the aortic incisions. Car-

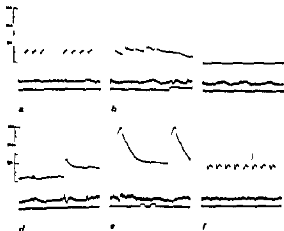



Fig. 1. Simultaneous tracings of the aortic pressure and lead II of the electrocardiogram in a normothermic dog. a, normal. b, effect of caval occlusion and at the signal the injection of acetylcholine into the coronary arteries. c, record during complete arrest. The undulations of the electrocardiogram are due to the mechanical respirator. d, shows the first ventricular contraction 10 sec. after the beginning of the coronary perfusion with oxygenated blood. e, improved contractions 4 min. later. f, normal pressure and electrocardiogram 30 min. later.

*From the Division of General Surgery, Henry Ford Hospital, Detroit, Mich. Supported by a grant from the Michigan Heart Association.

diac resuscitation in 5 animals was complicated by ventricular fibrillation which was corrected by 1 or 2 countershocks

During the period of massage the heart passed through a phase of cyanosis and right sided dilatation As massage was continued the cyanosis gave way to a rapid and dramatic spread of normal color over the heart, spontaneous contractions began and there was immediate emptying of the right side At this time the electrocardiogram showed the onset of a sinus rhythm We have since found that this period of myocardial cyanosis and lack of sinus rhythm can be reduced materially by intracoronary injection of small amounts of atropine

In March 1956 a pump-oxygenator of the type used by Lillehei, De Wall and their co workers became available to us[3] Since that time this method of protecting the brain has been used instead of hypothermia Following the tightening of the snares around the 2 vena caval cannulas the aorta was clamped between the inflow cannula (through a subclavian artery) and the coronary artery orifices A solution of acetylcholine (10 mg /kg of body weight) was injected into the proximal aorta and arrest of the heart was immediately produced The right ventricle was then opened and an interventricular septal defect created and repaired As the ventricular wall was being sutured the clamp was removed from the aorta, permitting the perfusion of oxygenated blood through the coronary arteries The first blood from the coronary sinus (containing acetylcholine) was removed from the right ventricle by aspiration Ventricular contractions frequently appeared within 1 min of the removal of the aortic clamp At first the contractions were co ordinated but weak and infrequent As the perfusion was continued, there was increase in the strength and frequency of the contractions and 4 or 5 min later the aortic pressure was sufficiently well maintained by the heart itself so that the pump oxygenator could be discontinued

Twenty five dogs have been operated on by this technique Cardiac resuscitation was achieved in all Ventricular fibrillation was encountered only 1 time and this was converted by 1 counter shock Ten animals expired in the first 48 hrs The cause of death in all appeared to be hemorrhage This cause of death has been eliminated by more careful attention to the neutralization of the heparin dose with protamine more meticulous care with the surgical hemostasis and careful washing and sterilization of the plastic tubes of the oxygenator apparatus

The longest period of arrest was 35 min This heart was resuscitated in the usual manner with resumption of ventricular contractions 50 sec after the release of the aortic clamp The pump-oxygenator was discontinued 1 min later Parts of the electro-cardiographic record are shown in Figure 2

A series of 8 children with interventricular septal defects has been operated on using the adjuncts of the pump oxygenator and induced cardiac arrest with acetylcholine As in the animals it was found that the intracardiac part of the operation was greatly facilitated The edges of the defects could be visualized accurately although in several instances it was necessary to divide 1 or 2 papillary muscles in order properly to retract a leaflet of the tricuspid valve The repair was reinforced with a pledget of Ivalon sponge in two cases

The behavior of the electrocardiogram in a typical case is shown in Figure 3

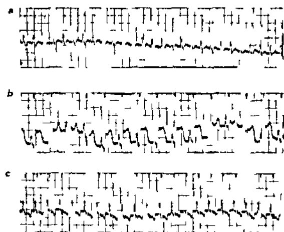



Fig 2 Lead II of the electrocardiogram of a dog during an experiment in which the heart was arrested for 35 min with acetylcholine a preoperative tracing b 5 min after the release of the aortic clamp (40 min after the beginning of the arrest) c Tracing taken 12 hrs later

In 6 of the 8 clinical cases of interventricular septal defect a normal sinus rhythm was established following the induced arrest In the other 2 cases ventricular fibrillation was significant One patient presented 2 episodes of fibrillation before the cannulation procedures in fact the subclavian cannula was inserted while the circulation was maintained by manual systole This heart (a very large one) resumed ventricular fibrillation following the closure of the defect and a regular rhythm was not established The heart of another patient fibrillated during resuscitation was controlled by countershock and a good aortic pressure was being produced but after the patient was beginning to recover consciousness the heart slowed down to standstill and intractable fibrillation occurred after massage Whether or not the fatal outcome in these 2 cases was related to the induced cardiac arrest or the pathologic conditions encountered and the suturing procedures cannot be stated with certainty Two of the patients in whom a sinus rhythm was established died in the immediate postoperative period of causes unrelated to the heart itself one had cerebral edema after improper cannulation of the superior vena cava and another had intrathoracic hemorrhage with inadequate blood replacement

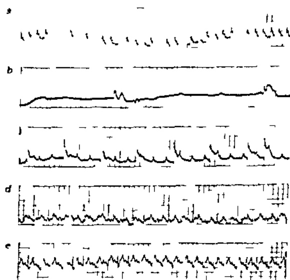



Fig 3 Lead II of the electrocardiogram of a patient having closure of an interventricular septal defect with the pump oxygenator and acetylcholine induced cardiac arrest a preoperative tracing b shows the appearance of the first ventricular contraction 1 min after the removal of the aortic clamp c 2 min after the release d four min after the release e 24 hrs after the operation

SUMMARY

Animal experiments have shown that acetylcholine is a safe and effective cardioplegic agent for the heart in both the hypothermic and normothermic states In a series of 8 open heart operations for the repair of interventricular septal defects induced cardiac arrest has appeared to be a valuable adjunct

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# METABOLIC CHANGES ASSOCIATED WITH THE USE OF THE MICRO BUBBLE TYPE PUMP OXYGENATOR UNDER NORMOTHERMIC AND HYPOTHERMIC CONDITIONS*

SAM E STEPHENSON JR JOHN L SAWYERS GEORGE L HOLCOMB FRANK GOLLAN ROLLIN A DANIEL JR AND H WILLIAM SCOTT, JR

In an experimental investigation of the clinical applicability of the combination of hypothermia and extracorporeal circulation numerous physiological biochemical and metabolic studies have been made These studies were undertaken in an effort to evaluate the physiological changes brought about by the use of an extracorporeal circuit in survival experiments under either hypothermic or normothermic conditions This report concerns data collected from a group of 150 animals subjected to cardio pulmonary by pass with or without ventricular cardiotomy Perfusion and oxygenation were accomplished by the micro bubble pump oxygenator as described by Clark Gollan and Gupta[1] or 1 of 4 other oxygenators designed by Gollan and based on this principle It was also hoped that these studies might reveal an explanation of certain deaths encountered in this series in which detailed gross and microscopic pathologic study failed to explain the death of the animal These unexplained deaths constituted over 50 per cent of the failures in this group of experiments

For this study blood samples were obtained for biochemical and formed element analysis preoperatively during perfusion at the completion of the experiment and daily for 3 days postoperatively These samples were an

*From the Department of Surgery and the Light Surgical Research Laboratories Vanderbilt University School of Medicine Nashville Tennessee This work supported by the United States Public Health Service Grant H 1997 (C)

alyzed for serum chloride potassium sodium, total serum protein with albumin globulin ratios, carbon dioxide combining power, non protein nitrogen sugar, calcium phosphate plasma hemoglobin, platelets, packed cell volume and white cell count

### METHOD

Unselected mongrel dogs were anesthetized with sodium pentobarbital Hypothermia, when used, was induced by the immersion technique lowering the esophageal temperature to 32°C The animal was intubated and attached to a modified Pneophore respirator during the cooling process A bilateral thoracotomy in the third intercostal space with transection of the sternum was used The animal was then prepared for cannulation and connection to the pump oxygenator after sodium heparin (5 mg /kg) was given intravenously Total cardio pulmonary by pass was carried out for a minimum of 30 min and ventricular cardiotomy when used, for a minimum of 15 min Total body perfusion was accomplished by using the Gollan pyrex or lucite pump oxygenator or the Sigmamotor pump with 1 of the 3 modified Gollan oxygenators * Body temperature was further lowered to 21 to 28°C in some of the experiments by the use of a cooling coil in the circuit The pump oxygenator in approximately one half of the hypothermia experiments was primed with mammalian Ringer s solution, no donor blood being necessary until completion of the perfusion

Postoperatively the heparin was neutralized by protamine sulfate after the method of Gollan Hamilton and Meneely [2] A number of the dogs were rewarmed by the immersion technique and the remainder by slow rewarming to a body temperature of 36°C Penicillin and streptomycin were administered for 7 days Small fresh whole blood transfusions were given to maintain a normal packed cell volume The dogs were allowed to eat and drink as soon as they reacted Three to 500 cc of 5 per cent dextrose/water was occasionally necessary on the first postoperative day, but in general no other electrolyte replacement was given

Perfusion flow rates in this series of animals varied rather markedly under hypothermia The earlier experiments were performed by attempting to pump physiologic cardiac outputs for the body temperature of the dog These values were calculated after the method of Bigelow [3] In the later experiments under hypothermia and in all the experiments under normal temperatures flows were calculated to be approximately 3 times the azygos factor as determined by Cohn and Fillcher [4]

### RESULTS

The data are tabulated and condensed into Table I which presents an average of many determinations for each study The preoperative immediate postoperative and first postoperative day averages only are presented for the sake of brevity These results show the major trends in all studies With the exception of the total serum protein and variations in the albumin

*The Clark Gollan pump oxygenator utilizes intermittent suction and pressure to circulate the blood as compared to the finger like compression of the blood conduits by the Sigmamotor pump The suction pressure is controlled by a simple electronic circuit containing 2 electro magnetic relays and energized by the perfusate coming in contact with positive and negative electrodes

*Table 1 Average Values of Biochemical Determinations*

| | HYPOTHERMIA | | | NORMOTHERMIA | | |
|---|---|---|---|---|---|---|
| DETERMINATIONS | PRE OP | POST OP | 1ST POST OP DAY | PRE OP | POST OP | 1ST POST OP DAY |
| Blood Sugar mg % | 95 | 91 | 80 | 96 | 90 | 82 |
| NPN mg % | 30 | 29 | 25 | 26 | 29 | 27 |
| Calcium mg % | 9.9 | 9.0 | 8.7 | 10.9 | 9.6 | 9.3 |
| Phosphorus mg % | 4.05 | 3.1 | 4.2 | 4.01 | 4.4 | 4.5 |
| $CO_2$ mEq/L | 22.0 | 17.4 | 18.9 | 23.4 | 16.2 | 19.5 |
| Chloride mEq/L | 102 | 114.9 | 103.8 | 104.7 | 110.1 | 105.5 |
| Sodium mEq/L | 144.7 | 145.5 | 140.2 | 145.3 | 146.6 | 145.0 |
| Potassium mEq/L | 3.4 | 2.7 | 3.4 | 4.0 | 3.0 | 3.8 |
| Total Serum Protein G % | 6.2 | 2.8 | 4.8 | 5.9 | 4.5 | 4.7 |
| Albumin G % | 3.2 | 1.4 | 2.4 | 3.5 | 2.4 | 2.8 |
| Globulin G % | 3.0 | 1.4 | 2.4 | 2.4 | 2.1 | 1.9 |

globulin ratio all values returned to the normal average range (± 1 standard deviation) within the first 3 postoperative days

From a study of Table 1 one sees that the blood sugar non protein nitrogen serum calcium and serum phosphate remain fairly constant both under hypothermia and at normal temperatures A further evidence of physiologic equilibrium is found in the sodium and potassium determinations There is an over all average increase in the serum sodium of 0.9 mEq/L and a decrease in the serum potassium of exactly the same amount The highest serum sodium recorded was 157.5 mEq/L and the lowest potassium 1.8 mEq/L A constant change in the serum chloride level was present however but with no significant difference in regard to body temperature differential There was a constant elevation of the serum chloride after perfusion averaging 8.1 mEq/L The variation in chloride ranged from a low of 85.9 mEq/L preoperatively and 99 mEq/L postoperatively to respective highs of 119.4 mEq/L and 123 mEq/L

The lowering of the carbon dioxide combining power is also constant in our study the average change being a little more severe at normal temperatures (7.2 mEq /L compared to 5.6 mEq /L) The over all average decrease of $CO_2$ was 6.23 mEq /L On the average all electrolyte studies are within the range of normal by the first postoperative day

The average drop in total serum protein with perfusion under hypothermia was 3.4 gm per cent compared with 1.4 gm per cent at normal tempera

alyzed for serum chloride potassium, sodium, total serum protein with albumin globulin ratios, carbon dioxide combining power non protein nitrogen sugar, calcium, phosphate, plasma hemoglobin platelets packed cell volume and white cell count

METHOD

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Perfusion flow rates in this series of animals varied rather markedly under hypothermia The earlier experiments were performed by attempting to pump physiologic cardiac outputs for the body temperature of the dog These values were calculated after the method of Bigelow [3] In the later experiments under hypothermia and in all the experiments under normal temperatures flows were calculated to be approximately 3 times the azygos factor as determined by Cohn and Lillehei [4]

RESULTS

The data are tabulated and condensed into Table 1 which presents an average of many determinations for each study The preoperative immediate postoperative and first postoperative day averages only are presented for the sake of brevity These results show the major trends in all studies With the exception of the total serum protein and variations in the albumin

*The Clark Gollan pump oxygenator utilizes intermittent suction and pressure to circulate the blood as compared to the finger like compression of the blood conduits by the Sigmamotor pump The suction pressure is controlled by a simple electronic circuit containing 2 electro magnetic relays and energized by the perfusate coming in contact with positive and negative electrodes

mize changes in the $CO_2$ content of the blood The average decrease of 6 23 mEq /l has not seemed of sufficient magnitude to require any corrective measures in the postoperative period

The most marked variation from normal in the biochemical studies was found in the total serum protein determinations and albumin globulin ratios This appears at first glance to be more marked with hypothermia Most of the hypothermic animals however were perfused with a pump oxygenator whose priming volume was 300 to 500 cc In most cases a large per cent of this volume was composed of mammalian Ringer s solution and in many dogs no donor blood was used in the actual perfusion The average decrease in protein of 3 4 gm per cent with hypothermia is therefore due in part to a simple dilution factor No definite cause has been found for the four fold loss of albumin over globulin

The most distressing change as previously observed by Gollan *et al* [2] was the marked reduction in white cells and platelets This drop was more profound with perfusion under hypothermia The 3 factors responsible for this are (1) the physiologic reduction in white cells and platelets which occurs with hypothermia (2) the dilution factor brought about by the use of Ringer s solution and (3) the large surface area necessary for defoaming in the micro bubble type pump oxygenator which traps numbers of these cells in the interstices of the nylon filaments These changes as mentioned before are rapidly reversed by the administration of fresh or decalcified whole blood

The unexplained deaths in this series of animals remain a mystery None of the alterations observed in this study can be incriminated as being of sufficient magnitude to account for the death of the animal

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*Table 2 Average Values for Formed Elements of Blood*

| | HYPOTHERMIA | | | NORMOTHERMIA | | |
|---|---|---|---|---|---|---|
| DETERMINATION | PRE OP | POST OP | 1ST POST OP DAY | PRE OP | POST OP | 1ST POST-OP DAY |
| WBC/m³ | 8 970 | 2,222 | 15 635 | 13 438 | 5 553 | 42 150 |
| Platelets/m³ | 230 000 | 91 000 | 167 000 | 210 666 | 39 739 | 196 000 |
| Plasma Hemoglobin | 5 mg% | 12 3 mg% | — — | 5 mg% | 33 mg% | — — |
| PCV | 38.5 mm | 26 4 mm | 39 mm | 39 mm | 32 mm | 40 mm |

tures This loss of serum protein was primarily at the expense of the albumin fraction irrespective of whether these animals were subjected to perfusion at normal or lowered body temperatures The amount of this albumin deficit was frequently great enough to cause a reversal of the albumin globulin ratio The average loss of total serum protein when the pump oxygenator circuit was primed with whole blood was 1 4 gm per cent the deficit consisting of a four fold loss of the albumin fraction over the globulin fraction This change has also been confirmed by electrophoretic patterns The animal usually corrected this protein deficit in the first postoperative week if a severe infection was not present

The second table shows the results of measurements of the formed elements of blood and of plasma hemoglobin determinations It is apparent from this table that changes in the formed elements of the blood are similar under each of the experimental conditions but that they are more marked with hypothermia *The use of fresh whole blood transfusions after* completion of the perfusion rapidly reversed these changes and samples of blood drawn 2 hrs after the completion of the procedure frequently yielded normal values There was a marked elevation of the white blood cell count on the first postoperative day which gradually returned to normal Total eosinophile counts in both the hypothermic and normal temperature groups revealed evidence of satisfactory adrenal function in the immediate and late postoperative period

The average plasma hemoglobin level of 12 3 mg per cent with hypothermia is not statistically different from the level of 33 mg per cent at normal temperatures as the hypothermic group is composed of a smaller *number of determinations*

DISCUSSION

In general these studies of the major cations anions proteins and formed elements of the blood in animals submitted to total cardio pulmonary by pass with micro bubble pump oxygenators have shown no significant changes which are not rapidly reversible irrespective of whether body temperatures were lowered or maintained at normal levels

The consistent elevation of serum chloride observed in the early postperfusion period in these experiments may be due in part to the addition of normal saline or Ringer s solution to the perfusate during the pumping procedure

In this study the gas mixture in the oxygenating chamber was composed of 95 per cent oxygen and 5 per cent carbon dioxide This seemed to mini

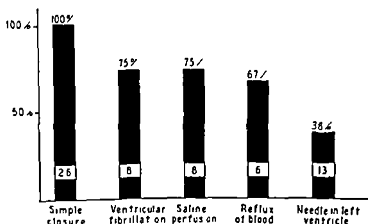



Fig 3 Incidence of air embolization using various techniques of closure Figures in light blocks indicate number of experiments in each group

cephalic trunk and a 7 mm T shaped cannula was inserted through a slit in the descending aorta The cannula in the ascending aorta allowed blood to flow into a glass bubble trap from which it emerged to enter the T cannula in the descending aorta By ligating the aorta between the 2 brachiocephalic trunks the left ventricular output was shunted through the bubble trap and returned to the descending aorta

Blood was drawn off by catheters in the venae cavae into the heart lung machine which consisted of a Sigmamotor pump and DeWall type bubble oxygenator The oxygenated blood was put back into the animal through the T cannula in the descending aorta

Figure 1 demonstrates the circulatory arrangement during creation and closure of the defect Figure 2 demonstrates circulation through the bubble trap after closure of the defect and cessation of artificial perfusion

RESULTS

Atrial septal defects were created and closed 61 times In no instance was air recovered from the aorta during the period that the heart lung apparatus was maintaining the animals cardio respiratory function The incidence of demonstrated air embolism with various techniques of closure is shown graphically in Figure 3

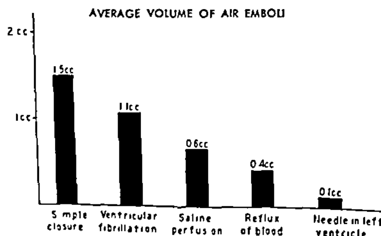



Fig 4 Average volume of emboli associated with various techniques of closure

# AN EXPERIMENTAL METHOD FOR MEASUREMENT OF AIR INTRODUCED INTO THE GREATER CIRCULATION DURING OPEN HEART CLOSURE OF ATRIAL SEPTAL DEFECTS

V L WILLMAN E C NEVILLE AND C R HANLON

The introduction of air into the systemic circulation during open heart closure of septal defects is a serious hazard The amount of air necessary to cause serious harm has been studied experimentally in dogs by Geoghegan and Lam [1] The incidence of air embolism associated with various techniques however has not been recorded nor has the quantity of air introduced been determined

This report describes an experimental method for measuring the amount of air introduced into the greater circulation during open heart closure of atrial septal defects using a heart lung apparatus The efficacy of various methods designed to avoid air embolism has been compared

## METHOD

Mongrel dogs of 10 to 15 kg weight were anesthetised with thiopental sodium With the animal supine the chest was entered through a bilateral fourth intercostal space trans sternal incision The azygos vein was ligated and the cavae encircled with umbilical tapes A 7 mm straight metal cannula was introduced into the ascending aorta through the right brachio

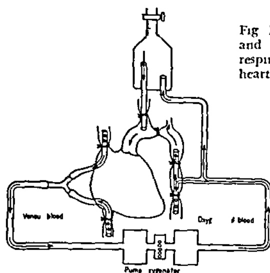



Fig 1 Circulatory arrangement during creation and closure of the atrial septal defects Cardio respiratory function is being maintained by the heart lung apparatus

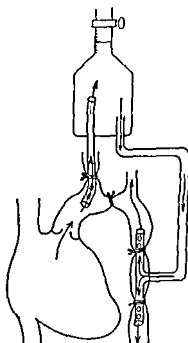


Fig 2 Circulation through the bubble trap after closure of the defect

### METHOD

Details concerning the Gibbon type mechanical pump-oxygenator and the recording equipment have been given elsewhere.[1, 2] Forty five patients of the group were selected for more particular study on a basis of weight and rate of blood flow obtained during perfusion, and an analysis of their records has shown certain related events to occur during and after the period of perfusion.

**Body Weight and Blood Flow During Perfusion.** For the 80 patients in whom these two variables were studied it was found that as the weight of the patient increased the flow in terms of cubic centimeters per kilogram per minute decreased. Since many factors influence the actual flow obtained during perfusion, the points were widely scattered, but a good correlation was observed when the patients were placed in groups for each 10 kg of body weight and the average flow for each group was plotted against the average weight (Table 1). Data obtained at preoperative cardiac catheterization showed a similar relationship between body weight and systemic flow in terms of cubic centimeters per kilogram per minute.

**Arterial Pressure and Blood Flow During Perfusion.** In all perfusions the aortic blood pressure varied directly with the rate of perfusion flow, and over the comparatively small range of flow (0.8 to 1.0 L./min.) which was available for study the relation appeared to be a linear one. In general the mean aortic blood pressure recorded during perfusion was lower than the values obtained in the preperfusion and postperfusion periods, even when the rate of blood flow during perfusion approximated the previously determined cardiac output of the patient. Consistently during and after the period of perfusion the normal relation of the central to the peripheral arterial systolic pressure was disturbed in that the central systolic peak exceeded the peripheral by 5 to 20 mm./Hg.

**Central Venous Pressure.** Central venous pressure varied directly with the rate of blood flow, but the actual pressure about which the changes occurred appeared to be related to the volume of blood within the patient. When venous pressure was plotted against the rate of blood flow several curves

*Table 1. Relation of Body Weight to Rate of Perfusion Flow*

| | WEIGHT OF PATIENTS IN KG | | | | | |
|---|---|---|---|---|---|---|
| | LESS THAN 10 | 10-20 | 20-30 | 30-40 | 40-50 | MORE THAN 50 |
| Cases in group | 13 | 27 | 15 | 5 | 6 | 14 |
| Weight, kg | | | | | | |
| Average | 5.3 | 16.2 | 24.5 | 33.3 | 47.8 | 59.8 |
| Range | 3 7 9 5 | 11 4 19 1 | 20.5 30 | 31 8 34.5 | 44-49.5 | 50.9 84 1 |
| Flow, cc./kg./min. | | | | | | |
| Average | 92.7 | 75.5 | 62 | 59.8 | 45 | 48.8 |
| Range | 42 220 | 47 126 | 25 104 | 41 90 | 20 91 | 30 88 |

It can be seen that embolization always occurred when the defect was simply sewed without precautions. Induced ventricular fibrillation, flooding of the atrium with saline solution, or allowing blood to fill the atrium just prior to final tightening of the suture line lowered the incidence of embolization slightly. Venting of the left ventricle by means of an indwelling 18 gauge needle at the apex lowered the incidence of embolization substantially.

Figure 4 compares these various techniques as to average volume of embolus. The size of the embolus is smallest when a needle is maintained as a vent in the apex of the left ventricle during the open heart procedure. This technique is similar to that described by Miller and co-workers.[2]


SUMMARY

A method is presented for evaluation of systemic air embolization by means of a bubble trap placed at the outflow from the left ventricle. Measurements were made *following creation and closure of atrial septal defects* using a heart lung apparatus.

Several common techniques designed to prevent air embolism were evaluated. Venting the left ventricle by means of a needle in the apex during closure considerably decreased both the incidence and volume of emboli.



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# STUDIES IN EXTRACORPOREAL CIRCULATION III. THE RELATION OF BLOOD FLOW AND BLOOD VOLUME DURING EXTRACORPOREAL CIRCULATION IN MAN*

DAVID E. DONALD AND EMERSON A. MOFFITT

Although a variety of mechanical oxygenators and blood pumps have been described for extracorporeal circulation and oxygenation of blood, two main approaches to the perfusion of the patient have been developed. In one the arterial pump is set to deliver a previously selected and constant amount of oxygenated blood to the patient, irrespective of the volume of venous blood delivered per minute from the patient to the apparatus. In the other the rate of return of oxygenated blood from the apparatus to the patient is governed by and is at all times equal to the rate of outflow of venous blood from the patient to the apparatus. It was under this second type of perfusion that the following observations have been made on 80 patients undergoing open intracardiac operations at the Mayo Clinic.

*The Mayo Clinic, Rochester, Minn., and The Mayo Foundation, Rochester, Minnesota (a part of the Graduate School of the University of Minnesota).

of this controlling volume of 50 cc Thus relatively small changes in the circulating volume of the patient machine circuit are reflected in significant changes in the rate of perfusion flow While it has proved impossible to quantitate the relation of the changes in flow to the changes in volume the knowledge that the withdrawal from the circuit of as little as 30 cc of blood will produce a measurable change in the rate of flow has proved invaluable in the proper maintenance of the perfusion and support of the patient

Studies at present in progress in the laboratory indicate that during perfusion in the dog a withdrawal of blood amounting to 10 per cent of the blood volume will reduce the rate of blood flow by almost 50 per cent and that flow will continue at this reduced value until the volume abstracted is returned

The direct relation of aortic and central venous pressure to flow the simultaneous response of both these pressure systems to alterations of flow the apparent inability of the patient to compensate for loss of blood and the marked sensitivity of the patient machine rate of flow to alterations in circulating volume have led to the belief that under conditions of extracorporeal circulation the patient behaves as a relatively noncompliant system If this be so then it follows that the rate of flow in the system will change with and be sensitive to alterations in volume within the system It is recognized that this concept cannot be verified in the operating theater but must be studied under more controlled conditions in the laboratory

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## A SIMPLIFIED PUMP OXYGENATOR WITH FLOW EQUAL TO NORMAL CARDIAC OUTPUT*

JEROME HAROLD KAY AND ROBERT A GAERTNER

At present there are only two practical methods of oxygenating blood spreading the blood in a thin film in an atmosphere of oxygen or mixing bubbles of oxygen in the blood Although the bubble oxygenators are efficient the difficulty of obtaining high flows the difficulty of complete removal of bubbles and the destruction of many of the constituents necessary for the clotting of blood are distinct disadvantages In all of the filming oxygenators an important factor is the amount of surface area of blood that must be exposed to the gas phase

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could be drawn for a single perfusion The curves were concave toward the pressure axis but the position of each curve on the graph seemed related to the blood volume of the patient since it lay at a higher pressure level when the blood volume was normal than when the blood volume was reduced as after a sudden loss of blood

A significant but unexplained finding was that when alterations occurred in the outflow from the apparatus to the patient the accompanying changes in aortic and central venous pressure were almost simultaneous in their time of occurrence

**Flow of Blood and Oxygen Saturation of Mixed Venous Blood** Data from 45 patients demonstrated the following points

1 In the individual patient the oxygen saturation of the mixed blood from the caval veins varied directly with the rate of perfusion flow

2 When the patients were placed in 2 groups on a basis of body weight (a) in each group the higher oxygen saturation of venous blood was associated with the higher flow and (b) at each level of venous oxygen saturation the group with the higher weight exhibited the lower flow in terms of cubic centimeters per kilogram per minute These points are illustrated in Table 2 which also indicates the fairly wide range of flow associated with each level of venous oxygen saturation

**Perfusion Flow and Alteration of Blood Volume** Proper to the use of the Gibbon type pump oxygenator is the concept that the patient determines his own rate of flow during the perfusion The technique of perfusion has been designed to establish conditions which enable the patient to deliver blood flow at a maximal rate to the machine

The experience gained with these 80 patients has created the impression that (1) alteration of blood volume is the principal factor affecting the rate of blood flow during perfusion and (2) under conditions of extracorporeal circulation the usual mechanisms whereby the patient compensates for loss of blood are severely depressed or in abeyance

The control system of the Gibbon type pump oxygenator makes this a constant volume machine in which input and output are constantly balanced and the apparatus itself operates through full scale on a controlling volume of 50 cc Since the volume of blood in the apparatus is constant and the patient is unable to respond in the usual fashion to a loss of blood a reduction in the circulating volume of blood in the patient is reflected in a reduction

*Table 2 Relation of Venous Oxygen Saturation to Perfusion Flow*

| GROUP | WEIGHT kg | VENOUS O SATURATION PER CENT 51 60 | 61 70 | 71 80 | 81 90 |
|---|---|---|---|---|---|
| Less than 20 kg | | FLOW cc /kg /min | | | |
| Average | 11 5 | 56 (1*) | 68 (8) | 87 5 (8) | 130.5 (7) |
| Range | 3 7 19 1 | | 45 111 | 60 147 | 72 220 |
| More than 20 kg | | | | | |
| Average | 44 1 | 26 5 (2) | 41 5 (8) | 53.5 (4) | 67 8 (7) |
| Range | 20 9 84 1 | 25 28 | 28 63 | 42 66 | 54 89 |

*Figures in parentheses indicate number of patients in each unit

establish the film, the blood was brushed on the screen. Samples were then obtained with this length at the desired flows. The rate of flow was adjusted by changing the height of the bottle of blood. A piece of screen was then cut off the bottom of the strip so that it measured the next shorter length to be tested. Samples were again obtained at the same flows. This procedure was repeated for each succeeding shorter length to be tested. The per cent saturation was plotted against the screen length and a curve was obtained for each flow rate.

Two types of screen were used in these experiments: (1) Saran window screen and (2) stainless steel screen used in the Gibbon oxygenator.

RESULTS

In a typical experiment the oxygen capacity was 18.1 vol. per cent and the saturation of the venous blood was 61.3 per cent. Three flow rates were used: 10, 20 and 30 ml./min. The shape of the curves was similar. The major part of oxygenation occurred over the first 20 cm. of screen length. Ten ml./min. appeared to be the optimum flow. At this rate and a screen length of 20 cm. the saturation was raised from 61.3 per cent to 91.1 per cent. This represented an increase of 30 per cent. With an additional 10 cm. of screen, the saturation was raised only 1.4 per cent and with a total length of 45 cm. of screen the saturation was 99 per cent or only 8 per cent more saturated than the blood at 20 cm. (Fig. 2). In this particular experiment Tyler #538 Type 304 Stainless Steel Ton Cap Screen was used. However, the results were the same whether the stainless steel or plastic screen was used.

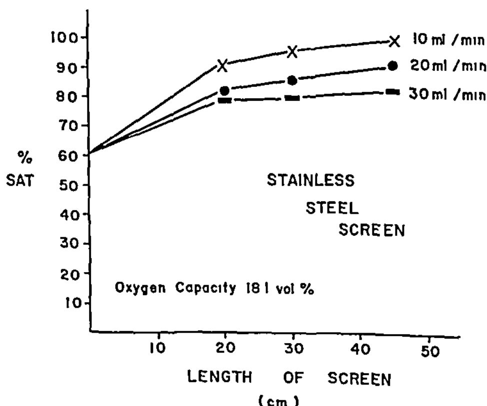



Fig. 2. Reveals the % oxygenation with various flow rates and various lengths of screen with a constant screen width (2.5 cm.)

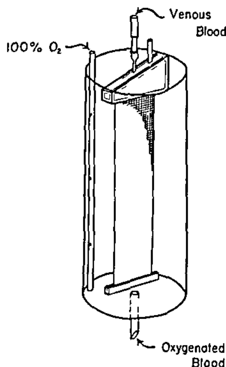



Fig 1 Apparatus used to determine the optimal screen length and rate of flow for a stationary screen oxygenator

After first determining the optimum length of screen and optimum blood flow on the screens a pump oxygenator of the Gibbon type was constructed and used successfully in dogs

METHOD

In order to determine the effect of screen length on the efficiency of the stationary screen oxygenator the following experiment was performed A single test apparatus was used for all the experiments (Fig 1) This consisted of a small plastic distributing chamber with an inlet for venous blood and an outlet for the removal of air From this distributing chamber a strip of screen 2 5 cm wide was suspended In order to add weight to the screen and thereby keep it straight 2 strips of plastic were clamped at its lower edge

The distributing chamber and attached screen were suspended in a plastic cylinder Ten liters per minute of 100 per cent oxygen were blown into this cylinder through a glass tube with 3 openings The openings were directed toward the wall of the cylinder instead of toward the film of blood so that there would be little or no turbulence produced in the blood phase by the jets of gas

Human blood of the same type was pooled for the experiments In order to simulate the conditions seen clinically the saturation of the venous blood was raised to between 35 and 60 per cent by swirling it in a flask in an atmosphere of 100 per cent oxygen Samples of oxygenated blood were collected in a small beaker under oil Oxygen contents were measured by the technique of Van Slyke and McNeil Carbon dioxide contents were not determined

For each experiment a strip of screen was attached to the distributing chamber This strip measured the greatest length to be tested in that given experiment The pooled venous blood of 35 to 60 per cent saturation was run into the distributing chamber with an intravenous drip set In order to

mal as well as the quantity of blood in the bottom of the oxygenator can be regulated by merely changing the speed of the arterial pump. During the perfusion period only the rate of the arterial pump is changed, and by maintaining a constant level in the bottom of the oxygenator the entire extracorporeal system is kept balanced. The coronary sinus blood is aspirated by gentle suction (10 cm. of water) into a reservoir (CR) and then flows by gravity into the venous reservoir.

Using this pump oxygenator in 10 consecutive animals total cardiac bypass was carried out for 35 to 45 min. In all of the animals the right ventricle was open for 30 min. and 8 of these animals were chronic survivors.

## SUMMARY

A very efficient simply designed and constructed pump oxygenator of the Gibbon type is described. This apparatus is capable of oxygenating and pumping 5 L. of blood per minute.

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# THE ULTIMATE IN VIVO SURVIVAL OF ERYTHROCYTES WHICH HAVE CIRCULATED THROUGH A PUMP OXYGENATOR*

Wilmer C. Hewitt, Jr., Ivan W. Brown, Jr., G. S. Eadie, Wirt W. Smith and W. C. Sealy

The recent increasing use of pump oxygenators in open cardiac surgery has resulted in much interest in the effect of such devices on the blood exposed to them. Up to the present, interest has focused mainly on their effect on the blood coagulation mechanism and also on immediate red cell hemolysis which, with the present pump oxygenators, it is agreed, does not occur to any great extent. Few if any studies have been carried out on the long term survival of red cells which have been circulated through an extracorporeal circuit. Evidence is now accumulating which points to deleterious effects arising at interfaces between blood and foreign surfaces or blood and oxygen bubbles. The following is a preliminary report of studies of *in vivo* erythrocyte survival as affected by pump-oxygenators.

## METHOD

The oxygenator employed in these studies has been described previously.[1] Briefly, it consists of a plastic bag containing 650 cc. of pure oxygen with additional capacity to accommodate 500 cc. of blood. As the venous blood enters from the patient, the bag oxygenator is gently rocked to and fro by

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DISCUSSION

**Apparatus** With the information from the above experiments an oxygenator was designed and constructed It is similar to that described by Gibbon except for the screens being shorter and wider than those of Gibbon The optimal length for oxygenation is approximately 25 cm and the optimal width for convenience is 10 cm If the screens are longer the extra length is wasted and this results in greater hold up of the blood and decreased efficiency of oxygenation

Since the maximum blood flow per 2 5 cm of screen width is around 20 ml /min in order to construct the oxygenator to adequately oxygenate 5 L of blood we used 610 cm of screen width This surface area was obtained by using 16 screens 40 cm in width and 25 cm in length

Only 2 pumps are required for the apparatus Those used are of the DeBakey non occlusive roller type Each pump compresses a single piece of Tygon tubing 0 5 inch in diameter The output of each pump is directly proportional to the revolutions per minute and ranges from 0 to 10 000 ml /min

The circuit is illustrated in Figure 3 One of the pumps is used to maintain a constant film on the screens and is in the internal circuit This internal circuit consists of the venous reservoir (VR) internal circuit pump (ICP) oxygenator (O) and the low resistance shunt from the bottom of the oxygenator to the bottom of the venous reservoir The internal circuit pump is set so that the flow is greater than the amount siphoned from the animal or returned to the animal As first described by Gibbon the internal circuit pump maintains a constant film of blood on the screens The quantity of blood on the screens therefore does not vary Venous blood is siphoned from the cavae and therefore any changes in venous return are automatically adjusted for by an immediate similar opposite change in the shunt from the bottom of the reservoir to the venous reservoir The arterial pump (AP) returns oxygenated blood from the bottom of the oxygenator through a filter (F) to the animal During total cardiac bypass the blood pressure of the ani

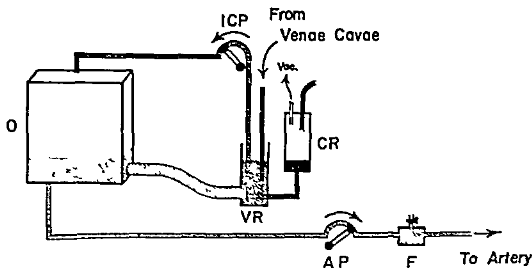



Fig 3 Schematic diagram of pump oxygenator (See text)

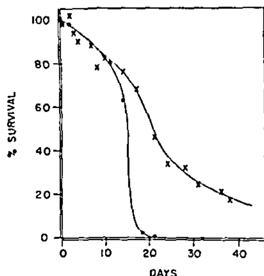



Fig 2 *In vivo* survival of red cells in normal recipients after passage through the pump oxygenator and patient s circulation during 2 human operations for open cardiac surgery lasting 24 min (x) and 2½ hrs () Tagging with $Cr^{51}$ Points corrected for radioactivity decay but not for elution of the isotope

was tagged with $Cr^{51}$ but also when it was tagged with Fe [9] In addition the final blood returned to the oxygenator at the close of 2 open cardiac operations on humans was tagged with $Cr^{51}$ and followed in healthy compatible recipients In both these cases there was a premature loss of the transfused erythrocytes the survival curves showing the same S shaped drop (Fig 2)

DISCUSSION

The disappearance curve of erythrocytes which have circulated through a pump-oxygenator and have been subsequently transfused into a normal compatible subject frequently shows after a short normal period of loss a sharp S shaped drop sometimes great enough as to indicate total disappearance of transfused cells within 30 or 40 days after transfusion instead of the normal 3 or 4 mo Whether this occurs or not appears to depend in part on the recipient for the same blood transfused into two recipients may show the loss in one and not in the other It is of course never seen with undamaged cells

The significance of this phenomenon is still far from clear The S shape of the curve seems to mean that the damage has been inflicted on all cells simultaneously and the length of time elapsing before the disappearance of the cells is apparently subject to the usual biological variation—a normal distribution about a mean The delay before the effect of the damage is seen may possibly be explained in one of several ways First is the possibility of an immune reaction Here we must assume that the trauma inflicted by the pump-oxygenator is such as to render the cells antigenic although the undamaged cells are not After a certain period—1 wk or more—antibody production has reached a level sufficient to cause removal of the affected cells It must also be assumed that in many cases only a fraction of the cells are traumatized to a degree that would render them antigenic and the removal of cells is therefore incomplete Such a theory can easily explain the delay but in this case the disappearance curve should be exponential rather than S shaped Another possibility is that the process causing disappearance of old cells ( senescence ) is accelerated in some way It is difficult to see how this could lead to an S shaped curve for it would be necessary to assume e g that the acceleration of senescence was less marked in the youngest cells as

hand so as to spread the blood over the bag's internal surface. In this way 500 cc. of blood can be fully oxygenated within 1½ and 2 minutes without foaming or frothing and with a minimum of trauma. Once oxygenated, the blood is pumped back into the patient's subclavian artery directly from the bag while the venous blood is entering another bag and being oxygenated.

The oxygenator is used with the dual Sigmamotor pump model T 6S. Except for the silicone rubber pump head tubes, the entire extracorporeal circuit is composed of non-wettable disposable polyvinyl chloride plastic which had been sterilized by autoclaving.

Whole venous blood was drawn from healthy donors into plastic bags containing 15 mg. of heparin for each 500 cc. of blood. In every case the blood was put through the pump oxygenator and transfused within an hour or 2 after withdrawal from the donor. Except in the human clinical cases, a sample of unpumped, unoxygenated blood was tagged and transfused into a healthy compatible recipient as a control. Blood grouping and compatibility tests, including indirect antiglobulin tests, were carried out in all instances on the blood of the donor and that of all recipients. Similarly, in dog experiments, animals were first grouped according to the ABCDE classification of Young *et al.*[2] and only compatible recipients chosen on the basis of cross-matching tests. In both human and animal experiments, sera of all recipients were retested at the end of the study for any demonstrable red cell antibodies which might have been provoked by the red cells transfused.

*In vivo* red cell survival was followed by tagging the cells with $Cr^{51}$ according to the method of Read.[3] The methods of counting radioactivity, et cetera, and plotting of data have been described previously.[4] Canine red cells were followed after tagging with both $Cr^{51}$ and $Fe^{59}$.[5] In these experiments the latter isotope was counted in a well-type scintillation counter.

## RESULTS

**Experiment 1.** Fresh heparinized blood from a healthy donor was recirculated through the pump oxygenator at a rate of 450 cc./min. Samples were taken before and after pumping. Each sample was divided into 2 equal aliquots, tagged with radiochromium, and each aliquot transfused into healthy compatible recipients. In about half of these (6 out of 11) there was an S-shaped drop in the survival curve occurring usually in 1 to 4 wks. after transfusion (Fig. 1). Similar results were seen in dogs not only when blood

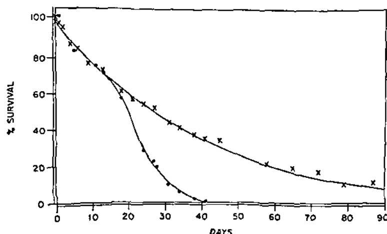



Fig. 1. Comparison of *in vivo* survival of red cells in normal recipients before (x) and after (·) passage through the pump oxygenator for 30 min. Tagging with $Cr^{51}$. Points corrected for radioactive decay but not for elution of the isotope.

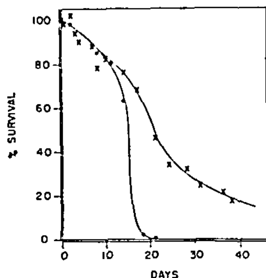



Fig 2 *In vivo* survival of red cells in normal recipients after passage through the pump-oxygenator and patient's circulation during 2 human operations for open cardiac surgery lasting 24 min (x) and 2½ hrs (•) Tagging with $Cr^{51}$ Points corrected for radioactivity decay but not for elution of the isotope

was tagged with $Cr^{51}$ but also when it was tagged with $Fe^{59}$ In addition the final blood returned to the oxygenator at the close of 2 open cardiac operations on humans was tagged with $Cr^{51}$ and followed in healthy compatible recipients In both these cases there was a premature loss of the transfused erythrocytes the survival curves showing the same S shaped drop (Fig 2)

## DISCUSSION

The disappearance curve of erythrocytes which have circulated through a pump oxygenator and have been subsequently transfused into a normal compatible subject frequently shows after a short normal period of loss a sharp S shaped drop sometimes great enough as to indicate total disappearance of transfused cells within 30 or 40 days after transfusion instead of the normal 3 or 4 mo Whether this occurs or not appears to depend in part on the recipient for the same blood transfused into two recipients may show the loss in one and not in the other It is of course never seen with undamaged cells

The significance of this phenomenon is still far from clear The S shape of the curve seems to mean that the damage has been inflicted on all cells simultaneously and the length of time elapsing before the disappearance of the cells is apparently subject to the usual biological variation—a normal distribution about a mean The delay before the effect of the damage is seen may possibly be explained in one of several ways First is the possibility of an *immune reaction* Here we must assume that the trauma inflicted by the pump oxygenator is such as to render the cells antigenic although the undamaged cells are not After a certain period—1 wk or more—antibody production has reached a level sufficient to cause removal of the affected cells It must also be assumed that in many cases only a fraction of the cells are traumatized to a degree that would render them antigenic and the removal of cells is therefore incomplete Such a theory can easily explain the delay but in this case the disappearance curve should be exponential rather than S shaped Another possibility is that the process causing disappearance of old cells ( senescence ) is accelerated in some way It is difficult to see how this could lead to an S shaped curve for it would be necessary to assume e g that the acceleration of senescence was less marked in the youngest cells as

well as those which had lived half the potential life span Such an *ad hoc* hypothesis is far from convincing More plausible perhaps is the suggestion that the trauma causes damage to the surface of the erythrocyte so that an enzyme system or systems is damaged in such manner that concentration of an essential metabolite within the cell slowly falls to a critical level

Since in the first experiment cells were labelled with $Cr^{51}$ the question arose whether the presence of this foreign metal played a role or even whether the whole phenomenon were not due to an increased rate of chromium elution rather than cell disappearance The fact that the same phenomenon is seen when cells are labelled with $Fe^{59}$ built into their hemoglobin seems to be sufficient evidence to refute such a suggestion

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## DESCRIPTION AND EVALUATION OF A ROTATING DISC TYPE RESERVOIR OXYGENATOR*


FREDERICK S CROSS ROBERT M BERNE, YOICHI HIROSE
RICHARD D JONES AND EARLE B KAY


It is the purpose of the present report to describe a rotating disc type reservoir-oxygenator which has been thoroughly evaluated in the laboratory and used in 30 clinical cases to date The essential components of this apparatus are a model TS Sigmamotor pump and a rotating disc type reservoir oxygenator The basic mechanism of the oxygenator is a series of 59 teflon coated stainless steel discs 0 4 mm thick and 12 2 cm in diameter, mounted 4 5 mm apart by means of stainless steel spacers on a central shaft The disc assembly is supported horizontally within a silicone coated pyrex cylinder 33 cm long and 13 3 cm in diameter by gasketed end plates of stainless steel likewise treated with silicone resin (Fig 1) Blood introduced at one end passes along the cylinder and is removed from the bottom of the opposite end The rotation of the discs effectively prevents channeling of blood along the bottom of the cylinder The oxygenator is primed with 1400 cc of whole blood which immerses the discs 4 1 cm This gives a thin film of blood of similar width on the surface of the discs to be exposed to oxygen as the discs

rotate With the oxygenator primed to 1100 cc the static exposed area of the 59 discs is 0.9 sq m At the generally used disc rotation rate of 120 RPM this gives an exposure area of 108 sq m /min The discs are rotated by a pulley mounted at one end of the shaft by means of a V belt and a 1/80 HP motor Oxygen and 1½ per cent carbon dioxide are supplied through a perforated stainless steel tube mounted within the cylinder above the discs The oxygen mixture is warmed by passing through tubing immersed in a sand bath heated by an electric mantle To prevent condensation within it the oxygenator is warmed by a wrapping of silicone rubber covered resistance wire the temperature of which is controlled by a variac All electrical connections are mounted on a platform over 5 ft above the floor to eliminate explosion hazards Provision is made at the inlet face of the oxygenator for filling prior to use the addition of blood to the cylinder during a run and for sampling the venous return to the oxygenator from the patient The output face is provided with openings to vent off the circulating oxygen and carbon dioxide for the collection of samples of oxygenated blood and to receive a thermo couple for the continuous monitoring of blood temperatures Coronary sinus blood has been collected by means of a sump type suction tip and pumped into a defoaming chamber at the inlet face of the oxygenator from which it is returned to the oxygenator No bubble traps or filters have been placed in the arterial line

The oxygenator can be dissembled easily into its component parts for cleaning It is sterilized in the autoclave after reassembly including the oxygen flow tube and the heating wire The inside is then dried by flushing with oxygen or air while still hot or by standing in the hot autoclave The connecting tubes are sterilized in zephiran for 24 hrs and then rinsed and mounted in the pump circuit The tubing is then rinsed with at least 2 L of saline solution before being connected to the patient

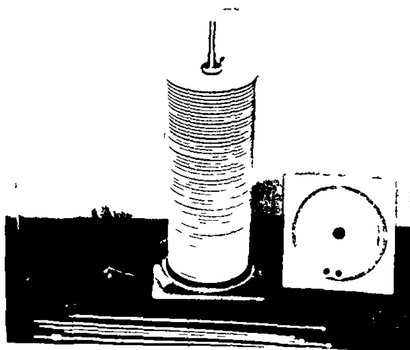


Fig 1 Reservoir oxygenator dissembled to show component parts

## METHOD

Effective pump oxygenators must maintain circulation as well as oxygenation during total cardiac bypass with a low morbidity and mortality rate To determine these functions of the present pump oxygenator both acute and survival experiments on animals were carried out as well as studies on cow blood from a local slaughter house Subsequently complete physiologic studies were carried out on the 30 clinical cases done to date These studies included accurate pre and postoperative weights continuous mean arterial blood pressures blood studies for oxygen and carbon dioxide content pH, plasma hemoglobin hematocrit platelet and cell counts, total proteins and A/G ratio, as well as electrolyte studies The cow blood studies were used primarily to evaluate the oxygenating capacity and efficiency of the oxygenator

## RESULTS

Mortality and morbidity were necessarily high in the developmental stages of the pump oxygenator However after the basic design and techniques were standardized a series of 20 dogs was obtained in which there were 2 deaths In 11 of the dogs simple cardiac bypass without cardiotomy was done with 1 death due to undetermined causes and in the remaining 9 dogs a ventricular cardiotomy was done with 1 death due to postoperative ventricular fibrillation following the institution of a citrated blood transfusion There have been no deaths attributable to the oxygenator *per se* in the first 30 human cases operated upon

Average mean arterial blood pressure obtained in 11 bypass experiments without cardiotomy ranged from 50 to 78 mm Hg About the same values were obtained in humans at flow rates from 40 to 115 cc /kg /min Patients were carried with mean arterial blood pressures as low as 43 for periods up to 32 min without apparent ill effect In perfusing both animals and humans it was frequently necessary to add neosynephrine or Vasoxyl to the oxygenator blood to prevent an initial blood pressure drop which frequently accompanied the start of the perfusion Attempts at maintaining high arterial pressures by increasing perfusion rates alone were not uniformly successful

A specific value as to the oxygenating capacity and efficiency of the present oxygenator has not been obtained With such factors as oxygen flow rates disc rate and blood temperature held constant the efficiency of the oxygenator varied not only with the blood flow rates but also with the degree of desaturation of the venous blood returning to the oxygenator With low oxygen saturations in the venous blood the oxygenator was more efficient in that more cubic centimeters of oxygen were added to the blood per minute but lower arterial oxygen saturations were obtained The reverse was true with higher levels of venous oxygen saturation (Table 1) In the cow blood studies the efficiency of the oxygenator ranged from 67 cc to 207 cc of oxygen per minute added to the circulating blood The arteriovenous differences ranged from 3 7 to 13 vol per cent depending upon the circumstances of the perfusion In humans arterial oxygen saturations ranged from 95 to 103 per cent except in 2 cases which were 92 and 93 per cent The venous oxygen saturation ranged from 33 to 58 per cent Table 2 illustrates a typical dog experiment

*Table 1 Oxygenation Experiments Using Cow Blood*

| | Blood Flow | Oxygen Saturation Vol % | | | cc $O_2$/min Added |
|---|---|---|---|---|---|
| Exp No | cc/min | Arterial | Venous | A V Diff | To Blood |
| 2 | 910 | 18 9 | 11 8 | 7 1 | 67 |
| 9 | 1550 | 17.5 | 11 7 | 5 8 | 78 |
| 17 | 2120 | 15 1 | 11 4 | 3 7 | 98 |
| 23 | 2860 | 15 8 | 11 8 | 4 0 | 114 |
| 24 | 3080 | 11 8 | 5 1 | 6 7 | 207 |
| 25 | 3080 | 16 1 | 11 8 | 4 3 | 132 |

*Table 2 Typical Dog Bypass Experiment*

| Weight | Blood Flow cc/min | Disc Rate RPM | $O_2$ Saturation Vol % Arterial | Venous | cc $O_2$/min Added To Blood |
|---|---|---|---|---|---|
| 74 | 1000 | 120 | 18 3 | 10.3 | 80 |
| | 1600 | | 18 9 | 10 1 | 141 |
| | 2000 | | 18 6 | 11 8 | 136 |

In the animal experiments hemolysis was not excessive The increase in serum hemoglobin over a 30 min pumping period ranged from 10 mg per cent to 160 mg per cent Variations in serum hemoglobin could not be related to such factors as disc speed up to 120 rpm or to pump speeds but hemolysis did increase with time At disc rates in excess of 130 rpm hemolysis was excessive and foaming in the oxygenator was present The average increase in plasma hemoglobin in the human cases during perfusion periods ranging from 12 to 103 min was 53 5 mg per cent The highest figure obtained was a plasma hemoglobin of 320 mg per cent most of which came from the sump suction

In neither the animal experiments nor human cases was there excessive destruction of platelets or other formed elements in the blood In 13 animal experiments the platelets dropped from an average figure of 184 770 to 96 000 or roughly one half of their pre perfusion value In humans the platelet counts remained above 120 000 in all but 4 instances In 10 clinical cases in which they were measured there was no significant change in the plasma fibrinogen or in clot lysis When protamine sulfate has been given in amounts equal to half the calculated dose of heparin and fresh citrated blood given postoperatively excessive bleeding has not been a problem, especially in the human cases

In the clinical cases done to date the pH of the arterial blood during the bypass period has ranged from 7 14 to 7 62 the majority being between 7 3 to 7 4 There has been no significant change in the carbon dioxide content during or after a run, when 1½ per cent carbon dioxide is added to the oxygen mixture in the oxygenator A slight metabolic acidosis existed during the perfusion period which quickly returned to normal spontaneously post perfusion All blood studies in the patients have continued to be entirely normal during the postoperative period

Blood balance during the operative procedure in the human cases was well maintained in that all but 3 of the 30 postoperative weights were within

¼ pound of the preoperative weights This has been corroborated by normal postoperative hemoglobin and hematocrit values

DISCUSSION

The pump oxygenator described is simple in design easy to operate and can be constructed to oxygenate over a wide range of blood flow rates without requiring excessive blood for priming purposes Although it resembles the Bjork[1] apparatus it differs in its general design the use of plastic discs and in its greater oxygenating capacity

Although the oxygenating capacity of the present apparatus has not been accurately ascertained, it probably has an upper limit of about 2000 cc /min A larger cylinder with a greater oxygenating capacity has been constructed for use in larger individuals and has been used clinically A smaller cylinder is being constructed for use in infants in order to reduce the amount of blood needed to prime the oxygenator The model used in the work to the present time is considered to be the basic experimental unit and continued modifications are being made along lines retaining the basic principles described

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# PREVENTION OF VENTRICULAR FIBRILLATION IN THE NORMOTHERMIC AND HYPOTHERMIC HEART III THE ADVANTAGE OF QUINIDINE OVER ATRIO CAVAL BLOCK*

SAM E STEPHENSON JR AND F BRACHLEY MAIN

The control of ventricular arrhythmia has assumed a role of major importance in a large number of surgical procedures At the present time ventricular fibrillation must be controlled or prevented for the successful outcome of procedures in three main surgical enterprises each of which is of major importance The adequate control or prevention of ventricular fibrillation could bring about the following changes

(1) The prolongation of the physiological safe period for total inflow outflow cardiac stasis in conjunction with hypothermia

(2) The clinical acceptance of the combined use of hypothermia and extracorporeal circulation for prolonged open cardiac procedures thereby utilizing the safety factors derived from each procedure

(3) A direct surgical approach on the coronary arteries for relief of arteriosclerotic occlusive disease

The various reported adjuncts to reduce cardiac arrhythmia were tried by us and failed to yield as satisfying results as have been reported

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Hyperventilation (Swan) increased $CO_2$ in respirator air (Nava & Lewis) and atrio caval block with Procaine (*Schumaker et al*) yielded some reduction in the incidence of ventricular fibrillation but left something to be desired

In the light of previous work by one of us, our experience was extended to hypothermia and a direct comparison of quinidine and atrio caval block was made

METHOD

The preparation was essentially that of Baley La Due and York [1] Unselected mongrel dogs weighing from 5 to 12 kg were anesthetized with 20 to 30 mg of pentobarbital sodium per kilogram of body weight Intermittent positive pressure or Pneophore type respirators were attached to the animals and a left anterio lateral thoracotomy was performed through the third intercostal space An 0 5 x 0 5 cm opening was made in the pericardium and the anterior descending branch of the left coronary artery was isolated at its origin from the left common coronary artery A ligature of #2 braided silk was passed under the artery for subsequent occlusion Constant recording electrocardiograms were taken on most dogs Special care was taken not to injure the accompanying veins and those dogs with injured veins were excluded from the series (>0 1%) At the proper time occlusion of the artery was accomplished by traction mediated through a rubber band sufficient to obliterate the lumen of the vessel but not to displace the heart Care was taken not to disturb the heart during occlusion or immediately after release

Drugs were administered through a cannula in the femoral vein starting 10 min prior to occlusion and extending through the occlusive period Hypothermia when used was accomplished through the immersion technique with temperature taken to 30–32°C on removal from the ice bath This yielded a maximum reduction of 26–28°C When atrio caval block was used it was necessary to modify the procedure to include a bilateral thoracotomy and transection of sternum A concomitant group of controls proved that this variation in incision had no effect on the incidence of arrhythmia or mortality in the acute experiment

RESULTS AND DISCUSSION

The early basis of the work to be presented was determined some years ago working in conjunction with the medical department of our hospital [2] The findings of that investigation were based on the experimental preparation of 499 animals to first determine the incidence of ventricular fibrillation on temporary occlusion of a major coronary artery and the incidence of fibrillation on release of the temporarily occluded artery and secondly to provide a two fold method to test the efficacy of various anti fibrillatory drugs or procedures This study subsequently was extended to include 661 animals and to evaluate 12 drugs (Table 1)

Although a number of drugs gave statistically significant protection during the period of occlusion or release quinidine and to a lesser extent apresoline gave a highly significant dual protection This protection of quinidine is significant even in the range of 15 mg /kg which is probably lower than the optimal dose Time so far has not permitted an evaluation of increasing amounts of quinidine at normal temperatures Numerous control experi

*Table I Ventricular Fibrillation—Normothermic Drug Evaluation*

| DRUG | NO. OF ANIMALS | FIBRILLATED DURING OCCLUSION | INCIDENCE DURING OCCLUSION | SURVIVORS | FIBRILLATED ON RELEASE | INCIDENCE AFTER RELEASE | SIGNIFICANCE DURING | SIGNIFICANCE AFTER |
|---|---|---|---|---|---|---|---|---|
| Control | 330 | 91 | 28% | 239 | 169 | 71% | | |
| Procaine 16 mg/kg | 19 | 0 | 0% | 19 | 14 | 74% | high | |
| Pronestyl 46 mg/kg | 70 | 8 | 11% | 62 | 36 | 58% | high | suggestive |
| Quinidine 15 mg/kg | 41 | 3 | 7% | 38 | 18 | 47% | high | significant |
| Xylocaine 16 mg/kg | 15 | 5 | 33% | 10 | 5 | 50% | | suggestive |
| C 1191 3 mg/kg | 16 | 3 | 19% | 13 | 10 | 77% | significant | |
| Pendiomide 3 mg/kg | 31 | 4 | 13% | 27 | 14 | 52% | high | suggestive |
| Trasentine 10 mg/kg | 16 | 3 | 19% | 12 | 9 | 69% | significant | |
| Paveril $PO_4$ 5 mg/kg | 30 | 15 | 50% | 15 | 9 | 60% | | |
| A11 5 mg/kg | 14 | 6 | 43% | 8 | 4 | 50% | | |
| Regitine 0.5 mg/kg | 29 | 10 | 34% | 19 | 7 | 37% | | significant |
| Apresoline 0.5 mg/kg | 30 | 5 | 17% | 25 | 11 | 44% | high | significant |
| Priscoline 3 mg/kg | 13 | 10 | 77% | 3 | 1 | 33% | | |

ments were interspersed among the drug groups to give an added check on the technique.

It is interesting to note some features of this earlier work which makes this a reliable procedure to test and compare various drugs.

1. The incidence of fibrillation on occlusion and on release of the occluded artery varied less than 2 per cent between the first 25 control preparations and the total 330 control animals.

2. The early work extended over a 5 yr. period and included some 15 operators. The control group varies less than 1 per cent among these different individuals. We, therefore, believe this to be a stable reproducible preparation.

3. All animals have been autopsied. In only 1 instance has thrombosis been encountered. The difficulty of continued occlusion therefore does not become a problem unless intimal damage occurs.

*Table 2 Ventricular Fibrillation—Quinidine vs Atrio caval Block*

| | GROUP | NO OF ANIMALS | FIBRILLATED DURING OCCLUSION | INCIDENCE DURING OCCLUSION | SURVIVORS | FIBRILLATED ON RELEASE | INCIDENCE ON RELEASE |
|---|---|---|---|---|---|---|---|
| NORMOTHERMIC | Control | 330 | 91 | 28 | 239 | 169 | 71% |
| | Atrio caval Block | 10 | 1 | 10% | 6 | 4 | 67% |
| | Quinidine 15 mg /kg | 41 | 3 | 7% | 38 | 18 | 47% |
| HYPOTHERMIC | Control | 10 | 1 | 10% | 6 | 6 | 100% |
| | *Atrio caval* Block | 10 | 6 | 60 | 4 | 3 | 75% |
| | Quinidine 30 mg /kg | 10 | 0 | 0% | 10 | 0 | 0% |

The control of ventricular fibrillation under hypothermia is much more difficult its incidence being reported in various series of experiments from 5 per cent to 75 per cent of preparations The incidence of fibrillation increases markedly with further lowering of body temperature

The present work consisted of studying 4 new groups of animals numbering 10 dogs each (Table 2) First atrio caval block with xylocaine was compared with the control group and with quinidine at normothermic conditions This failed to demonstrate any perceptible protection from atrio caval block either during occlusion or on release

A group of control experiments under hypothermia revealed that all animals developed arrhythmia either during occlusion or on release When atrio caval block and quinidine were compared under hypothermic conditions the dual protective effect of quinidine was again far superior to the other procedure In this admittedly small series there has not been an episode of ventricular fibrillation when the dog has received quinidine in doses of 30 mg /kg of body weight

We believe that both atrio caval block with xylocaine and quinidine tends to slow conduction and lengthen the refractory period of the heart thus rendering the heart less susceptible to fibrillatory stimuli whether they be hypothermic mechanical anoxic or metabolic in nature In combatting all these stimuli quinidine in our hands has been superior to all other drugs or procedures tested

This work has been extended to the 3 previously mentioned fields of endeavor all experimentally and some clinically The administration of quinidine intravenously to animals extends for 3 to 4 min the maximum length of time that total inflow outflow stasis can be retained under hypothermia without development of ventricular arrhythmia

An additional beneficial effect has been observed by us using quinidine in conjunction with hypothermia in the experimental animal In a consecutive group of 16 animals under hypothermia there was a 57 per cent incidence of fibrillation and a 19 *per cent resuscitation rate* while in a comparable group

*Table 1 Ventricular Fibrillation—Normothermic Drug Evaluation*

| DRUG | NO. OF ANIMALS | FIBRILLATED DURING OCCLUSION | INCIDENCE DURING OCCLUSION | SURVIVORS | FIBRILLATED ON RELEASE | INCIDENCE AFTER RELEASE | SIGNIFICANCE DURING | SIGNIFICANCE AFTER |
|---|---|---|---|---|---|---|---|---|
| Control | 330 | 91 | 28% | 239 | 169 | 71% | – | |
| Procaine 4.6 mg/kg | 19 | 0 | 0% | 19 | 14 | 74% | high | — |
| Pronestyl 4.6 mg/kg | 70 | 8 | 11% | 62 | 36 | 58% | high | suggestive |
| Quinidine 15 mg/kg | 41 | 3 | 7% | 38 | 18 | 47% | high | significant |
| Xylocaine 4.6 mg/kg | 15 | 5 | 33% | 10 | 5 | 50% | | suggestive |
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| ATI 5 mg/kg | 14 | 6 | 43% | 8 | 4 | 50% | | |
| Regitine 0.5 mg/kg | 29 | 10 | 34% | 19 | 7 | 37% | | significant |
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# THE EFFECT OF ATRIOVENTRICULAR NODAL BLOCKADE ON FIBRILLATION PRODUCED BY ELECTRIC SHOCK*

WATTS R WEBB

Many factors are known to influence the development of ventricular fibrillation in the mammalian heart These include various drugs such as the sympathomimetic and parasympathomimetic agents electrolytes especially the ratio of calcium and potassium hormones temperature the status of the sino auricular node and the oxygenation and perfusion of the myocardium The present study has observed the effect of anaesthetization of the atrio ventricular node on ventricular fibrillation produced by faradic stimulation The role of the atrioventricular node and the Purkinje system in ventricular fibrillation has been little studied though Scherf Shaffer and Blumenfeld[1] have suggested that ventricular fibrillation is of multifocal origin with innumerable sustaining centers many of which may be located in the Purkinje fibers

## METHOD

Healthy mongrel dogs of both sexes weighing between 9 and 15 kg were anesthetized with pentobarbital Respiration was maintained by an intermittent positive pump respirator through an endotracheal tube The right chest was entered through the fourth interspace and the pericardium widely opened anterior to the phrenic The atrioventricular node is located approximately 1 cm cephalad to the coronary sinus orifice along the base of the posterior leaflet of the tricuspid valve at the junction of the atrial and ventricular septa The ventricular septum at this point was probed by a small gauge needle inserted through the right atrial wall When the A V node or the conduction bundle is stimulated arrhythmias are produced and at this point approximately ½ cc of 1 per cent procaine was injected If a satisfactory block was not produced other injections were made but usually the procaine was limited to a total of 5 cc.

In 8 dogs the sino auricular node which is located at the junction of the right auricle with the superior vena cava was blocked with procaine

A monitoring EKG was recorded to verify the nature of the rhythm produced The ventricles were stimulated with a 180 volt alternating 60 cycle

* From the Department of Surgery University of Mississippi Medical Center Supported by National Heart Institute Grant No H 2806

Of 48 animals receiving quinidine there was an 18 per cent incidence of fibrillation and a 100 per cent resuscitation rate. It, therefore, appears that quinidine should be a valuable adjunct to successful cardiac resuscitation under hypothermia.

Very little difficulty has been encountered from toxic reactions to quinidine experimentally or clinically in these low dosage experiments. The EKG on some animals receiving quinidine will show an occasional ventricular premature contraction. This has, however, been of no consequence.

Our only toxic manifestation clinically was seen in a 2[illegible] year old white male who was to undergo a direct approach to the pulmonary valve under hypothermia. Soon after the injection of quinidine (75 mg./kg.) he developed an idioventricular rhythm. This, however, returned to a normal sinus rhythm after a 45 min. delay. He subsequently tolerated inflow stasis of 12 min. duration for the correction of pulmonic valvular stenosis without arrhythmia. Some minutes following release, however, he developed cardiac arrest but was successfully resuscitated. He expired 8 hrs. following surgery after he had been awake and shown no evidence of neurological damage. Post mortem examination revealed other multiple congenital cardiac abnormalities.

The addition of quinidine (20–40 mg./kg.) to the perfusate in the experimental use of the combination of extracorporeal circulation and hypothermia has reduced the incidence of fibrillation in prolonged cardio-pulmonary bypass from 100 per cent in the early work of Gollan *et al.* to approximately 10 per cent at present. Various consecutive groups of experiments including up to 45 animals have been done without a single episode of ventricular fibrillation developing. It has also been possible after administering quinidine as reported by Gollan to cool dogs to 0°C. and produce cardiac arrest with recovery on rewarming without the development of ventricular fibrillation. This work has been done without hyperventilation or changing the content of carbon dioxide in the inspired air.

Experiments are now in progress to evaluate the protective action of quinidine for the direct surgical approach to the coronary arteries. The work at present is very promising and will constitute a future report.

SUMMARY AND CONCLUSIONS

1. A method is presented which affords a dual test of anti-fibrillatory drugs and procedures based on 530 control normothermic animals.

2. Under normal temperatures low doses of quinidine give the most highly significant dual protective action. [illegible] also shows promise but needs still further investigation.

3. [illegible] with xylocaine under normothermic or hypothermic conditions does not statistically alter the development of ventricular fibrillation from the control series under the conditions of this experiment.

4. Quinidine offers a very highly significant dual protective effect against ventricular fibrillation under hypothermia as tested by temporary coronary artery occlusion.

5. The use of quinidine experimentally and clinically has offered a marked reduction of ventricular arrhythmia under hypothermia alone or combined with extracorporeal circulation and has significantly increased the percentage of successful resuscitations.

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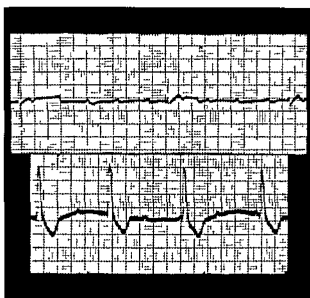


Fig 1 Top EKG Dog 33 showing complete atrioventricular dissociation with A V nodal rhythm Bottom EKG Dog 29 showing idioventricular rhythm

current which in innumerable previous experiments has invariably fibrillated the normal dog s heart

### RESULTS

Of the 8 dogs who had the S A node blocked with procaine as demonstrated by loss or depression of the P wave and marked slowing of the heart 4 developed ventricular fibrillation with faradic stimulation

On application of the faradic stimulus the 8 dogs which had procaine injected into the region of the node without producing the A V dissociation instantly developed persistent ventricular fibrillation as did 4 dogs which had no injections

A successful block of the atrioventricular node was produced in 22 dogs giving complete dissociation of the auricles and ventricles with an auricular rate of 140 to 180 and a ventricular rate of 24 to 60 Three of the dogs developed an A V nodal rhythm while in 19 an idioventricular rhythm developed which arose distal to the A V node presumably in the Purkinje fibers

In the 3 dogs with an A V nodal rhythm fibrillation occurred in one It seemed that some protection was offered here in that multiple stimuli were required to produce fibrillation

In the 19 dogs with an idioventricular rhythm ventricular fibrillation was not produced in a single instance by the faradic stimulation Likewise simultaneous stimulation on each side of the heart with 2 pairs of electrodes did not produce fibrillation in these circumstances

*Table 1*

| BLOCK OF | RHYTHM | DOGS | FIBRILLATION | PER CENT |
|---|---|---|---|---|
| — | S A | 12 | 12 | 100 |
| S A node | Auricular | 8 | 4 | 50 |
| A V node | A V nodal | 3 | 1 | 66 |
| A V node | Ventricular | 19 | 0 | 0 |

DISCUSSION

This finding that anesthetization of the atrioventricular node increases the resistance of the ventricle to fibrillation suggests a role of the atrioventricular node in the production and maintenance of fibrillation It is interesting that complete exclusion of the A V node is required for this full effect as procainization of the conduction system at the S-A node with the production of an auricular rhythm appears to offer some protection but less than that where an idioventricular rhythm is produced

Weidmann[7] has demonstrated that perfusion of cocaine and other antifibrillatory drugs stabilize the Purkinje fibers and thereby protect the isolated fiber from fibrillation It is not believed that this is the mechanism of protection here where the anesthetic has been given locally rather than by perfusion Those animals with procaine injected into the auricular and ventricular septa but not directly into the conducting system did not enjoy the protection received by those with a similar or less amount of procaine injected only millimeters away

It has been suggested that the protection offered by the block is by preventing vagal effect However if anything vagal stimulation should offer some protection as Hoff and Nahum[3] demonstrated that acetyl $\beta$ methyl choline chloride offered marked protection to the ventricles against fibrillation following electric shock

Hoff and Nahum[3] demonstrated that removal of the stellate ganglia and the adrenal glands enormously decreases the susceptibility of the heart to ventricular fibrillation following electric shock Supporting work for this has been presented by Harris and Bisteni[4] who found sympathetic ganglionic blockade drugs to reduce the ectopic activity following ligation of the anterior descending coronary artery There is of course the possibility that blockade of the A V node removed some of the sympathetic stimuli However as the sympathetic nerves do reach the ventricle directly as well as through the conduction system it does not appear that their influence has been completely removed by the block performed above

Shumacker and co-workers found that anesthetization of the S A node gave marked protection from ventricular fibrillation in hypothermic dogs subjected to right ventriculotomy and manipulation of the interventricular septum either at relatively low or moderate degrees of hypothermia They found a similar protection from dorsal sympathectomy or from the sympathetic blockade drugs but did not explain this phenomenon It is interesting that S A blockade does not give the degree of protection from electrical stimulation offered by an A V blockade

The exact mechanism of this particular process by which anesthetization of the A V node affords protection from ventricular fibrillation has no definite explanation at present Further studies of both the electrical phenomena and varying electrolyte concentrations may be of help

SUMMARY

1 Procaine anesthetization of the atrioventricular node in dogs producing complete heart block gives protection from ventricular fibrillation from faradic stimulation

2 Blockade of the S A node offers a lesser degree of protection

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# CORONARY FLOW AND RESISTANCE IN THE DOG DURING TOTAL BODY PERFUSION*

RAYMOND C READ JOHN A JOHNSON AND C WALTON LILLEHEI

The development of extracorporeal pump oxygenator systems[1,2] which allow temporary bypass of the heart and lungs during intracardiac surgery has also provided valuable experimental methods for the investigation of the circulation in different organs under conditions of controlled cardiac output The purpose of this report is to present studies of the coronary circulation in the dog during body perfusion at various flow rates

## METHOD

Ten small mongrel dogs averaging 9 kg in weight were selected for use in these experiments so that a wide range of body flow rates could be studied conveniently These animals were anesthetized with intravenous sodium pentobarbital

For these acute experiments a modified pump oxygenator[4] was utilized consisting of arterial and venous finger pumps† activating ½ rubber tubing connected with plastic adapters and ¼″ Mayon‡ polyvinyl tubing to the bubble oxygenator Oxygenation was accomplished by bubbling 100 per cent oxygen into the mixing tube of the oxygenator Antifoam spray§ was added in small amounts to the debubbling chamber The debubbling reservoir rested in a warming bath which maintained the temperature of the blood

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†Sigma Motor Company Middleport New York

‡Mayon Plastics Company Hopkins Minnesota

§Antifoam A Dow Corning Company Midland Michigan

and of the animal constant throughout the experiment. The apparatus was primed with 500 to 700 cc. of fresh heparinized donor blood. The venous pump was connected to the animal via plastic tubing and a #11 French polyethylene catheter inserted into both superior and inferior venae cavae through the right jugular vein. The holes in this catheter were so placed as to lie peripheral to the right atrium. Arterialized blood was returned to the animal through a similarly sized catheter with a terminal orifice introduced proximally for about 3 cm. into the right common carotid artery. Ventilation was carried out with a positive pressure respirator connected to a tracheal cannula. The chest was opened through a longitudinal sternal splitting incision. Both venae cavae were looped near the right atrium with umbilical tapes and the azygos vein was tied. The pericardium was opened and the main pulmonary artery was dissected from the aorta and looped with a tape. Arterial perfusion was started and the tapes around the venae cavae were secured on the venous catheter, thus diverting the venous return into the oxygenator. The venous pump had been started and adjusted to balance the outflow from the oxygenator reservoir. Soon after cardiac bypass had been started, a right angled glass cannula was passed through an incision in the main pulmonary artery into the outflow tract of the right ventricle and secured there. The outflow of this system was allowed to drain through plastic tubing led into a vertical reservoir. Coronary sinus and Thebesian venous drainage into the right heart thus collected accounts for approximately 95 per cent of the total coronary flow.[3]

The coronary flow was measured by the slope of the pressure change at the bottom of the coronary outflow reservoir. A constant slope is indicative of a constant flow rate, and the steeper the slope the higher the flow rate. All values obtained were the mean of 1 to 5 determinations. Mean aortic and vena caval pressures were measured through polyethylene catheters inserted into the appropriate femoral vessels. Right ventricular pressure was measured with a needle tip catheter inserted through the myocardium. All recordings were made on a Sanborn polyviso. The coronary reservoir was evacuated into the oxygenator when necessary with another SigmaMotor pump. In some animals the heart was fibrillated and defibrillated electrically.

The total body perfusion rate was determined at the beginning and end of each experiment by direct calibration of the arterial pump utilizing a graduated cylinder and stop watch. The output of this arterial pump was not influenced by the resistance offered by the inflow catheters nor by varying vascular resistance in the animal. During the actual experiments arterial inflow rates also were measured with a Ludwig stromuhr. Excellent agreement between the two methods was obtained.

## RESULTS

The total body perfusion was maintained for approximately 3 hrs. in each animal. Thirty two different flow rates were studied ranging from 7 to 81 cc./kg. body wt./min. The inferior vena caval pressure during perfusion varied between 3 and 8 mm. Hg, and in each case was not significantly different from the control readings. The pressure in the right ventricle was less than atmospheric when there was a siphonage effect created in the drainage tubing. At low (less than 20 cc./min.) coronary flows, however, the pressure was the hydrostatic head to the level of the sternum from the heart. This

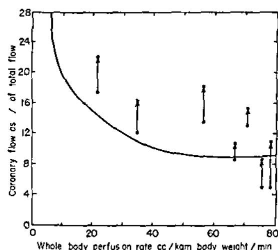



Fig 1 The average relationship between percentage coronary flow and perfusion rate as well as the effect of ventricular fibrillation at 7 particular flow rates

right ventricular pressure ranged around 5 mm Hg and has been disregarded in the values for pressure difference across the coronary bed

The pressure flow relationship of the coronary bed was essentially linear with an intercept on the pressure axis of about 18 mm Hg There was a tendency for the coronary vascular resistance to decrease during perfusion The proportion of the total body flow passing through the myocardium varied with the perfusion rate (Fig 1) Thus at high perfusion rates (80 cc/kg/min) the mean coronary flow made up 8 per cent of the total and at low rates (10 cc/kg/min) 24 per cent of the total flow passed through the myocardium However this relative increase was insufficient to prevent an absolute decline in flow at the lower perfusion rates

Induced ventricular fibrillation in 7 instances increased coronary flow the change being abolished on defibrillation (Fig 1) When coronary vascular resistance was plotted against blood flow a hyperbolic relationship was seen with markedly increased values at very low flows and higher flow rates being associated with declining resistance (Fig 2) The resistance showed a similar asymptote to infinity at low arterial pressures but a minimum was reached at slightly higher pressures with much higher pressures being related to an increase in resistance

At autopsy the mean heart weight was found to be 1 per cent of the average body weight

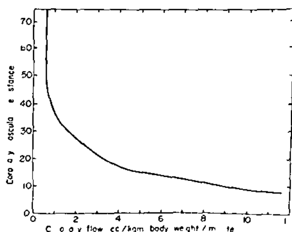



Fig 2 The average relationship between coronary flow and coronary vascular resistance units = $\frac{\text{Blood Pressure mm Hg}}{\text{Coronary Flow cc/kg body weight/min}}$

DISCUSSION

These studies show that under conditions of total body perfusion the bypassed heart receives a remarkably liberal coronary flow. When total body flow is reduced or if perfusion is prolonged the proportion of blood passing through the myocardium increases favoring the heart over other body organs. The flow through the essentially non working heart is comparable to that accepted (65 cc /100 gm heart weight/min )[4] for the normally functioning organ. This finding as well as the increase in flow associated with the presence of ventricular fibrillation are possibly explained by decreased intramural tension due to the absence of either normal function or contraction. This extravagant perfusion of the bypassed heart accounts for its excellent reaction to low flow body perfusions without increased susceptibility to ventricular fibrillation or other detectable damage. This tolerance has been observed repeatedly in the experimental and clinical experience with the azygos or low flow principle.[5] This favoring of the heart during extracorporeal maintenance of the circulation is in direct opposition to the effect of total circulatory standstill where the heart unless resuscitated becomes the limiting factor in survival.[6] The finding of a finite pressure gradient across the coronary bed when the flow has fallen to zero supports the hypothesis of Burton.[7] The minimal resistances at very low body flows suggest that hypoxia may begin to affect the heart at that time and this factor with accompanying accumulation of metabolites doubtlessly contributes to the decreasing resistance after prolonged perfusion.[8]

SUMMARY

1 The coronary circulation has been studied in 10 dogs whose hearts were bypassed with artificial maintenance of the circulation for in most cases 3 hours using a pump oxygenator with perfusion rates of between 7 and 81 cc /kg /min

2 Coronary venous return was drained from the main pulmonary artery and monitored continuously using an electromanometer

3 The relationship between arterial pressure and coronary flow was approximately linear with an intercept of 18 mm Hg on the pressure axis

4 The portion of the total body flow passing through the coronary bed varied between 1 and 27 per cent the higher proportions being associated with lower body perfusion rates

5 Coronary resistance tended to decrease with duration of perfusion and in 7 instances was also uniformly lowered by the induction of ventricular fibrillation

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# RAPID DETECTION OF CARDIAC ARREST WITH ELECTRICAL RESUSCITATION*

K WILLIAM EDMARK AND HENRY N HARKINS

Cardiac arrest is now the chief cause of operating room mortality [1] As surgeons we are justifiably concerned For operative mortality to continue to decrease methods for rapid detection and treatment of cardiac arrest must be developed

It is impossible to predict in which patient cardiac arrest will develop [2] It is most commonly seen in a healthy patient who is undergoing a major abdominal procedure Cardiac arrest is not a major cause of mortality in the poor risk patient with cardiac irregularities in whom it would be suspected Most commonly it is seen in a 61 year old male undergoing major abdominal surgery who is in good health and would be expected to survive the operation uneventfully

As such it would seem logical to direct attention toward developing a method whereby cardiac arrest could be instantly detected in the routinely anesthetized patient When detected resuscitation could be started at once without the unknown time delay between its onset and recognition that exists today In previous work[3] a simple cardiotachometer was developed for *monitoring the QRS complex by using 2 electrodes taped to the chest* A built in minimum rate warning alarm automatically sounded if the heart rate dropped below a predetermined level Using this apparatus to detect the cardiac arrest this experiment was undertaken to determine if the arrested heart could be resuscitated without cardiac massage using in its place an electrical stimulation applied across the axis of the heart through the unopened chest The stimulating pulse used for creating artificial ventricular contraction is 5 to 10 milliseconds in duration 100 volts in intensity and applied at a rate of 100 to 120 pulses per minute The same electrodes that operate the cardiotachometer are used for applying the stimulation across the chest

*From the University of Washington School of Medicine Department of Surgery This study was aided by a grant from the Washington Heart Association

To simulate the type of cardiac arrest that is seen in the operating room mongrel dogs were given a uniform dose of intravenous pentobarbital (26 4 mg /kg of weight) In addition to this a varying amount of ether was administered through an intratracheal tube The intratracheal tube was clamped and after cardiac arrest had occurred, transthoracic electrical stimulation was applied to the chest If ventricular contraction as manifested by a femoral pulse tracing did not occur coincidentally with the applied stimulation within 1 min, a conventional cardiac massage was done

Figure 1 illustrates the type of 4 channel continuous tracing made on each animal The tracing speed was one tenth of the normal rate for a cardiogram or 2.5 cm /sec From analyses of these tracings the following information was obtained Cardiac arrest was produced 45 times, 28 animals (65 per cent) were resuscitated by use of the transthoracic stimulation and the remaining 17 animals (35 per cent) required cardiac massage In this group death occurred in 2 animals The first died on the second postoperative day from anoxic brain changes The second developed a spontaneous ventricular fibrillation following cardiac massage Transient defibrillation was accomplished but could not be maintained The animal died 1 hr after the onset of cardiac arrest The over all survival rate in this series was 95 per cent

The stimulation as applied across the chest did not produce ventricular fibrillation In 1 animal the needle electrode was deliberately introduced through the chest wall into the myocardium Ventricular fibrillation was

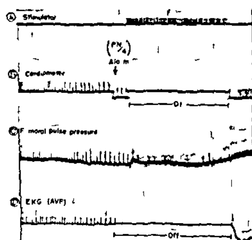



Fig 1 Typical 4 channel tracing showing A Application of cardiac stimulator pulse across the chest at 130 beats per minute B Cardiometer* indication Drop of base line indicates point at which the minimum rate warning device sounds (in this case at 40 beats per minute) and the respirator is started When stimulator is turned on the Cardiometer automatically stops functioning C Femoral pulse tracing At the irreversible rate point (I N/4) the spontaneous heart rate is 40 with a low pulse pressure Under the influence of the stimulator the heart rate instantly becomes 130/min and the pulse pressure gradually rises over a 1 min interval At the end of this interval when the stimulator is turned off the spontaneous heart rate has become 120 D AVF EKG tracing at 2.5 cm /sec The instrument used has a capacity input requiring it to be turned off while the stimulation is applied

*Trademark Physio Control Co

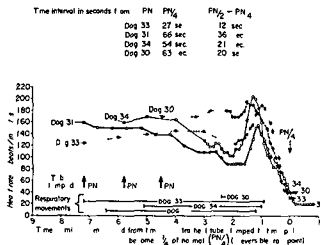



Fig. 2. Spontaneous respiratory movements are not seen in all animals as soon as the respirator is stopped and intratracheal tube clamped. Those deeply anesthetized and well oxygenated do not develop spontaneous respiratory movements for several minutes. Spontaneous respiratory movements stop as the heart rate reaches its maximum. This is followed by a precipitous decrease in heart rate. Time intervals PN – PN/4 and PN/2 – PN/4 are measured after the pulse rate maximum has been reached.

instantly precipitated as seen on the monitoring oscilloscope. Thoracotomy with defibrillation and uneventful recovery followed.

In studying the pulse rate changes that occurred prior to cardiac arrest a fairly typical pattern developed. Figure 2 shows a composite graph of the pulse rate of 4 animals. It is to be emphasized that the pulse rates indicated on this graph were determined by differentiating succeeding 6 to 12 sec intervals.

The time differential was chosen carefully to give the greatest meaning to the graph. A time differential in seconds (dx) that is too great yields a rate differential in heart beats a minute (dy) that is too small to have significance; for example

$$\begin{aligned}&\text{Lim } dy = 2\\ &dx \longrightarrow 30\end{aligned}$$

A pulse rate change of 2 beats a minute has little clinical significance. Conversely, if the time differentiation becomes too small, the smallest rate differential detectable becomes so large as to become confusing rather than helpful. When

$$\begin{aligned}&\text{Lim } dy = 30\\ &dx \longrightarrow 2\end{aligned}$$

we would know if the heart rate was 0, 30, 60, 90, 120, etc. But it is desirable to know the intermediate rates and therefore the following differentials were chosen:

$$\begin{aligned}&\text{Lim } dy = 10\\ &dx \longrightarrow 6\end{aligned} \qquad \begin{aligned}&\text{Lim } dy = 5\\ &dx \longrightarrow 12\end{aligned}$$

This yields detectable rate changes that have clinical meaning, namely 5 to 10 beats per minute.

The most significant finding was the extremely rapid fall that occurred in the pulse rate just prior to cardiac arrest. This pulse rate change varied between 110 and 240 beats per minute and the principal change occurred during the last 30 sec. By preliminary experimentation it was shown that when the heart rate dropped below one fourth of the normal rate, spontaneous resuscitation did not occur without a brief period of artificially induced ventricular contraction produced either by transthoracic electrical stimula

tion or cardiac massage Since in all cases spontaneous respiratory movements stopped prior to this point being reached it was concluded that when the heart rate reached one fourth of normal (PN/4) a 100 per cent mortality would result in the animals without both artificial respiration and artificial ventricular contractions being produced This point is arbitrarily called the irreversible rate point PN/4

The slope of the line of this decreasing heart rate when plotted on a log log scale gives a linear relationship with time during the 30 sec preceding the irreversible rate point At the irreversible rate point a slope change occurs which is a break with the rate time offering a mathematical correlation with the clinical observation that this point initiates cardiac arrest

### SUMMARY

Cardiac arrest was produced 45 times in mongrel dogs Artificially induced ventricular contraction was produced by electrical stimulation in 28 of these and the remainder required cardiac massage The pulse rate changes in the last 30 sec prior to cardiac arrest occur at a very high rate, and to be comprehended a cardiotachometer must be used Respiratory failure occurs as the pulse rate reaches a maximum preceding the precipitous pulse rate decrease When the pulse rate decreases to one fourth of normal spontaneous recovery does not occur without a brief period of artificially induced ventricular contraction This artificially induced ventricular contraction can be produced in 65 per cent of cases by electrical stimulation without cardiac massage If electrical stimulation is not effective within one minute artificial ventricular contraction must be produced by cardiac massage

### CONCLUSIONS

The pulse rate change curve immediately preceding cardiac arrest when produced by excessive anesthesia and anoxia is a characteristic one When the spontaneous heart rate has reached one fourth of normal in the absence of spontaneous respiratory movements cardiac arrest is present To be accurately detected at this earliest point a cardiotachometer with a minimum rate warning device should be used When cardiac arrest is detected at this point 65 per cent of the animals can be resuscitated without the necessity of cardiac massage by using a proper type of electrical stimulation

The mortality from cardiac arrest in experimental animals is much lower than that which exists in the operating room This difference in mortality is probably due to the unknown time delay that exists between onset of cardiac arrest and its actual detection The routine use in the operating room of a cardiotachometer with a minimum rate warning device would eliminate this delay

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# DIFFERENTIAL CARDIAC WARMING DURING HYPOTHERMIA*

JAMES R JUDE, JEAN P FAUTEUX AND LULU M HAROUTUNIAN

The heart is less tolerant of anoxia than the rest of the body under moderate hypothermia Lack of coronary blood flow is therefore a limit of the length of safe circulatory occlusion The cold heart is also extremely sensitive to manipulation with ventricular fibrillation not infrequently the result It can be postulated that if during general body hypothermia the heart were near normal temperature and the coronary blood flow maintained these problems would be obviated

This investigation is of a technique of coronary perfusion and differential cardiac warming utilized to achieve this state

## METHOD

Fifty mongrel male and female dogs weighing 8 to 15 kg were used Anesthesia was by endotracheal ether intermittent positive pressure with alveolar $CO_2$ maintained near 4 vol per cent and blood pH thereby normal

The animal was wrapped in a special blanket† through which ice water was circulated As cooling proceeded a thoracotomy was made sterilly through the right fourth interspace The aorta was isolated and a purse string of 3 0 arterial silk placed in its ascending portion 1½ cm distal to the aortic ring and proximal to any branches except for the coronaries

The coronary perfusion apparatus consisted of 500 cc of heparinized arterial blood (20 mg heparin) which had been freshly drawn from a healthy donor animal and kept at a constant temperature of 38 C The perfusate bottle was attached at a height sufficient to deliver a pressure of 75 mm Hg at the level of the ascending aorta In some experiments the regular transfusion tube was attached to a coil of polyethylene tubing (volume 10 cc) contained in a basin of water at 38 C From this differential warming basin the tubing ran a short distance directly to a #15 needle (short bevel with a 1 cm shaft) which was inserted through the purse string into the ascending aorta (Fig 1)

At 26–28°C the cavae were occluded and the aorta cross clamped distal to the perfusion needle The coronary perfusion was begun and a 4 cm right ventriculotomy or auriculotomy immediately performed Myocardial temperatures were taken with a #27 needle containing a thermostruhr at its tip The interventricular wall was either massaged with the fingertip or stimulated with a suture Close notice was made and recorded of the manner in which the heart recovered function after 10 to 20 min occlusion If no indication of return of cardiac function was evident in 3 min massage and resuscitation were initiated

## RESULTS

A right atriotomy was performed with differential cardiac warming by coronary perfusion on 8 dogs No ventricular fibrillation occurred and all

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*Department of Surgery The Johns Hopkins Hospital and University Baltimore Maryland This study supported in part by funds provided under Contract AF 41 (657)30 with the U.S.A F School of Aviation Medicine Randolph Field Texas

†Fabricated and provided by David Clark Mfg Co Worcester Mass

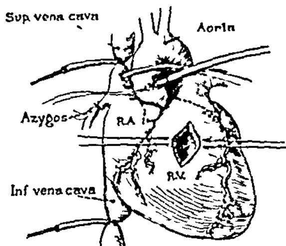



Fig 1 Method of coronary perfusion and differential cardiac warming during hypothermia

animals survived rewarm Five dogs were long term survivals The return of cardiac function on the re establishment of the circulation was good in every instance A good return of cardiac function was considered to be a blood pressure of at least 50 mm Hg with a good pulse pressure within 1 min after the opening of the cavae A poor return was one which would require 3 min or more to reach this state

Differential cardiac warming by coronary perfusion with a right ventriculotomy was performed on 11 dogs Ventricular fibrillation occurred in only 1 animal and that with the performance of the ventriculotomy With the coronaries being perfused the animal was readily reverted to a normal rhythm with a single electroshock after a 3 min period of fibrillation and without the use of massage or drugs The 1 unsuccessful dog in this group was due to right heart failure on the return of circulation after 17 min caval occlusion Ten animals were long term survivals

Thirty one control animals had a standard 10 to 11 min caval occlusion with a 4 cm right ventriculotomy The aorta was not occluded Eleven animals suffered ventricular fibrillation either with the ventriculotomy or during the occlusion period The heart did not resume its function and suffered right heart failure in 6 dogs Seven dogs had a poor and slowly returning cardiac function on the re establishment of the circulation All except 5 of those 24 animals succumbing to right heart failure or ventricular fibrillation were resuscitated by the use of massage drugs and electroshock as needed Only 7 dogs had a good return of heart beat and blood pressure without assistance

With a coronary perfusion pressure of 75 mm Hg gravity there was utilized 25 to 30 cc of perfusate blood per minute The gravity flow was completely selective being greater or less as dictated by the myocardial need

On perfusion with normothermic blood without the heat exchanger coil the myocardial temperature was elevated to 30 to 31°C With the use of the heat exchanger the myocardial temperature was elevated to 34 to 36 C There was little difference in the temperatures of the various portions of the heart

In general the entire procedure (cooling occlusion and rewarm) required about 2 to 2½ hrs and the animals were awake and standing in their cages within a few hours of completion The animals received no antibiotics and no parenteral blood or fluids

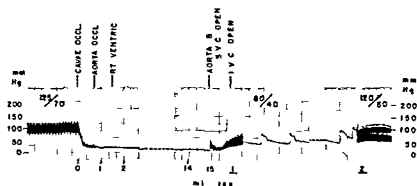



Fig 2 Effect of coronary perfusion with warm blood on return of cardiac function at 28 C Fifteen minute occlusion

Selective Coronary Perfusion
Dog 158 28 C

There was no indication of central nervous system damage in any of the animals rewarmed after circulatory occlusions of up to 20 min

## DISCUSSION

Coronary perfusion with differential cardiac warming reduced the incidence of ventricular fibrillation during the cardiac occlusion practically to zero in this series Cookson and Costas Durieux[1] and Shumway *et al*[2] have also emphasized the use of coronary perfusion during occlusion to prolong the possible period of cardiac occlusion

The culpability for ventricular fibrillation occurring on re establishment of the circulation can not be placed on the hypothermia *per se* as the heart both cold and more or less hyoxic depending on the length of circulatory occlusion may not accept the work load suddenly thrust upon it and right sided heart failure results Attempted resuscitation on the irritable myocardium then almost invariably results in ventricular fibrillation This fibrillation is secondary to heart failure and not directly due to cold The effect on the return of cardiac function of coronary perfusion with warm oxygenated blood is shown in the blood pressure and pulse tracing of Figure 2 as compared to that without coronary perfusion or cardiac warming in Figure 3

Selective coronary perfusion of normothermic blood by gravity pressure has been utilized here because it offers arterial blood to the coronaries when the myocardium requires it at a near normal pressure and temperature Our




Fig 3 The return of cardiac function in the control dog at 28 C Eleven minute occlusion

initial experiments in which we used a finger pump to supply the coronary perfusion gave an alarmingly high incidence of fibrillation The cause was found to be due to widespread myocardial hemorrhage Arteriovenous oxygen studies revealed that high coronary flows were not necessary in the cold empty beating heart With selective coronary perfusion 25 to 30 cc /min was sufficient flow

Clinically the use of a perfusate needle in the ascending aorta would allow the decision for use of coronary perfusion in any particular case to be delayed and yet rapidly be applied if desired without additional dissection Adequate flow is available through a modified #15 needle

Since hypothermia *per se* is of added risk to the operative procedure it is advisable to rewarm as quickly as possible Presently conventional methods of cooling and rewarming require good blood flow for heat conduction inward or outward A prompt and effective return of blood pressure following occlusion has the beneficial effect of enabling rapid elevation of the temperature

This method has been successfully used clinically by Dr Henry T Bahnson in closing an atrial septal defect with anomalous venous return in a 7½ year old male Myocardial irritability was absent and there was immediate return of excellent cardiac function

SUMMARY

Selective coronary perfusion of warm arterial blood reduced practically to zero the incidence of both ventricular fibrillation during cardiac occlusion and cardiac failure on re establishment of normal circulation

In 19 dogs with coronary perfusion and a right auriculotomy or ventriculotomy of 10 to 20 min duration at 26 to 28°C there occurred only one instance of ventricular fibrillation which was readily reverted to a normal rhythm Only 1 dog did not have an immediate return of good cardiac function

In 31 control dogs with a right ventriculotomy for 10 to 11 min at 25 to 28°C there were 11 instances of ventricular fibrillation In addition 13 dogs had either no or poor return of cardiac function

*We are grateful to Dr Alfred Blalock for his encouragement and advice in this study

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# *Coronary Arteries and Great Vessels and Angiography*

## STUDIES IN VENTRICULAR FIBRILLATION IN THE PIG EVALUATION OF AN ANTIFIBRILLATORY AGENT TESTED BY CLOSED CHEST CORONARY ARTERY OCCLUSION*

JOSEPH J GARAMELLA LYLE J HAY AND JAMES G ANDERSEN

*In spite of the vast amount of experimental work that has been performed* on coronary artery disease there still remains a great deal of confusion and controversy relative to the benefits of various revascularizing procedures and medications Experimental work has been dubiously accepted or refuted sometimes simply ignored principally because of the wide discrepancies reported on the consequences of acute coronary artery ligation in the dog our heritage dating to 1698 when this procedure was first performed by Chirac[1]

The mortality differences reported following coronary artery occlusion are readily understood if one will briefly survey the multiple variables present in a seemingly simple experiment These include 1) experimental animal (usually the dog)—health age size 2) premedication 3) anesthetic agent—respirator—pH 4) operator 5) site of ligation 6) period of open chest, 7) period of follow up i e time of death 8) season 9) temperature of animal 10) diet 11) coronary artery pattern i e left or right preponderance, 12) intercoronary development magnitude

With the object of minimizing the influences of premedication anesthesia and the open chest upon experimental acute coronary artery occlusion a reliable method of closed chest coronary artery occlusion has been developed Furthermore because of the variability of the coronary artery anatomy in the dog the present studies were performed on the pig The anatomical factors rendering the pig more suitable for this type of study have been given by Blumgart *et al*[2]

It is therefore the purpose of this report to present first a method of acute closed chest coronary artery occlusion in the pig and second the application of this method in testing an antifibrillatory agent Ro 2 5803† Ro 2 5803 is 2 6 Bis(1 piperidylmethyl)-4 (α α dimethylbenzyl) phenol dihydromide (Fig 1) According to Schallek and Wong[3] Ro 2 5803 is more toxic than quinidine in rats and mice it is more potent than quinidine in the isolated (rabbit) auricle test and equipotent in preventing auricular fibrillation in dogs

*From the Jay C Phillips Research Laboratory of Mount Sinai Hospital and the Department of Surgery University of Minnesota Minneapolis Minnesota Supported in part by the Minnesota Heart Association American Heart Association Cardiac Research Committee Minneapolis Minnesota National Heart Institute (Grant 2610) of the National Institutes of Health Public Health Service Fund for Research on Coronary Heart Disease University of Minnesota

†Made available for this study by Dr M J Schiffrin Hoffmann LaRoche Inc Nutley N J

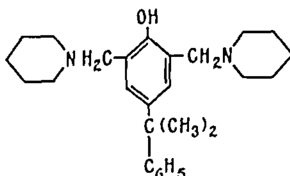



Fig 1 Chemical structure of Ro 2-5803

### METHOD

Young stockyard pigs weighing between 13 6 and 30 kg were anesthetized with intraperitoneal chloral hydrate 300 mg /kg of body weight and intravenous nembutal 3–4 mg /kg of body weight Animals were intubated and ventilated with compressed air by an automatic respirator Using aseptic technique left thoracotomy was done through the fourth or fifth interspace and the pericardial sac was opened At the bifurcation of the left coronary artery the left anterior descending branch was dissected free The coronary artery occluding apparatus was installed at the origin of the left anterior descending branch This mechanism* consists of a plastic snare which is passed around the coronary artery with ample slack to avoid compression of the vessel The relationship of the snare to the axis of the vessel is maintained by a plastic platform which overrides the vessel to be occluded and which is sutured to the adjacent epicardium An adjustable armature attached to the plastic platform can be passed under the main pulmonary artery and sutured to the adventitia for stabilization The plastic cord is enclosed within a flexible plastic cable which passes from the coronary artery through the thorax and is carried to the exterior between the ribs muscles subcutaneous tissue and finally through the skin where a control unit is fixed in position The control unit can be implanted under skin muscle or in the thorax Manual self winding or magnetically controlled movements of the control side of the occluding apparatus provide either acute one staged or chronic progressive vascular or tubular occlusion (Fig 2)

In these experiments 1½ to 21 hrs after installation of the occluding apparatus and chest closure acute one staged occlusion of the anterior descending branch of the left coronary artery was performed Electrocardiograms were taken before during and after coronary artery occlusion

### RESULTS

In 15 successful control studies this method of closed chest coronary artery occlusion was performed All 15 pigs developed irreversible ventricular fibril

*Minneapolis Honeywell Regulator Company

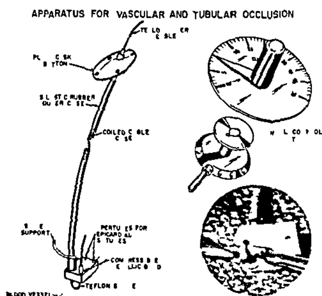



Fig. 2. Schematic representation of apparatus for acute and chronic occlusion. Inset (right lower corner) shows the character of vascular occlusion from a removed specimen.

lation confirmed by electrocardiography within 1½ to 27½ min. These results are in keeping with previous studies.[2]

In 18 pigs given Ro 2 5803 (0.57 to 8.8 mg./kg. of body weight) intravenously 5 to 9 min. before acute closed chest coronary artery occlusion, 10 developed ventricular fibrillation within 2 to 4½ min. Of the remaining 8 pigs, 7 survived and were sacrificed 1, 1, 6, 7, 8, 12 and 53 days following coronary occlusion. The 8th pig in this group was last seen alive 2½ hrs. following acute coronary occlusion and was found dead in the kennel the following morning; extensive bilateral pneumonia was found at autopsy. The survival times of control and treated pigs are summarized in Figure 3. In autopsies of all animals in the control and treated groups complete occlusion of the left anterior descending branch was confirmed in each instance. All the hearts of animals dying of ventricular fibrillation, both control and treated pigs, were contracted and hard.

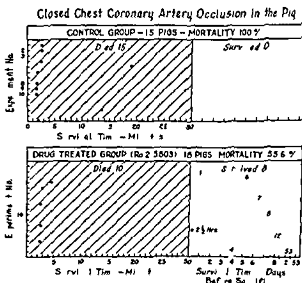



Fig. 3. Survival times of the control group and the drug treated group are charted.

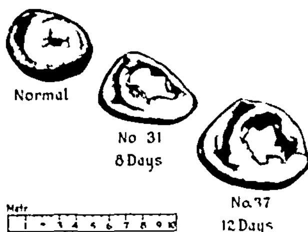



Fig 4 Transverse sections of pig hearts are shown Note the uniform thickness of the ventricular wall and septum in the normal heart Specimens number 31 and 37 were removed from drug treated pigs which had survived acute closed chest coronary artery occlusion of the left anterior descending branch of the left coronary artery The ventricular myocardium including the anterior portion of the septum supplied by this vessel demonstrates marked thinning and necrosis

The hearts of the drug treated pigs surviving the acute coronary occlusion and subsequently sacrificed showed in each instance extensive transmural infarction in the area of distribution of the occluded vessel (Fig 4)

Some effects of the drug noted prior to coronary occlusion include 1) tranquilizing change constant 2) tachypnea 3) hypotension 4) pupillary dilatation 5) electrocardiographic alterations (tachycardia pulsus alternans bigeminal rhythm sinoauricular block atrioventricular block T wave changes S wave changes) Optimum dosage toxicity and mechanism of action of the drug have not been established

## SUMMARY

1 A simple method of closed chest coronary artery occlusion is presented

2 Fifteen control pigs subjected to closed chest acute coronary artery occlusion died of ventricular fibrillation in 1½ to 27½ min (100 per cent) whereas in 18 pigs treated with antifibrillatory agent Ro 2 5803 8 survived (55 5 per cent mortality) These differences are statistically significant at the one per cent level

3 Further study relating to dosage toxicity and mechanism of action is indicated

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# THE EFFECTS OF GRADUAL OCCLUSION OF THE CORONARY ARTERIAL CIRCULATION IN DOGS AND PIGS*

ALBERT B. LOWENFELS, CHARLES G. NEUMANN, WILLIAM H. WADE, JERROLD VON WEDEL, JERE W. LORD, JR., AND J. WILLIAM HINTON

In connection with a previously described operation for revascularization of the heart with a pedicled skin flap, a new method for producing gradual coronary arterial occlusion was evolved.[1] A small stainless steel spring clamp with jaws separated by surgical gut was placed around the coronary artery. After a period of time the gut was slowly absorbed allowing the clamp to occlude the coronary artery.

It was felt that this clamp might provide a useful method to study the effect of chronic obliteration of the coronary arterial circulation. Naturally occurring coronary collaterals might be expected to develop during the gradual occlusion which would afford considerable protection to the heart when closure finally became complete. Others have carried out somewhat similar investigations using different methods of occlusion.[2, 3]

The authors thought that the use of delayed, self closing clamps would give insight into the amount of occlusion which a heart unprotected by any surgical method could withstand. It was felt that the mortality following gradual occlusion of a major coronary artery might be appreciably less than the figure of 70 per cent reported by Beck and Leighninger for ligation of the anterior descending branch of the left coronary artery.[4]

## METHOD

The clamp used was constructed of hard tempered stainless steel wire SMO 18 8 type 302 having a diameter of 0.025 in. This type of steel has been shown to be non reactive in the tissues. The wire was used to fashion an 8 coil spring which activated the clamp. The bends in the remainder of the clamp were placed with a set of hand pliers. In the fully closed position, the clamp will withstand a pressure of 300 mm. Hg and prolonged exposure to the arterial pulsation was found not to weaken the spring mechanism.

This clamp was applied around a blood vessel with the jaws of the clamp held apart by a bolus of surgical gut. After contact with the body fluids the gut was slowly absorbed and the jaws of the clamp occluded the vessel. Fifty turns of 00 plain surgical gut were used in most instances, although in a few clamps 00 chromic gut was used to separate the jaws.

It has been found that when the clamps were buried in the pericardial sac they closed in a period of approximately one month.

In the canine experiments, healthy adult mongrel dogs weighing from 10 to 20 kg. were used. The animals were anesthetized with nembutal using 30 mg./kg., intubated, and placed in the lateral position with the left side uppermost. Ventilation was maintained with a mechanical insufflator and room air.

An incision in the fourth intercostal space was used to expose the heart. The pericardium was opened and a Blalock clamp placed on the auricular

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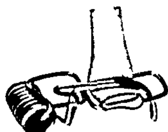


Fig. 1 Photograph of self closing delayed action arterial clamp The upper picture shows how a bolus of surgical gut keeps the jaws of the clamp apart The lower picture reveals how the jaws close around a simulated blood vessel after absorption of the gut The scale is in centimeter




appendage to give better exposure of the base of the left coronary artery This was dissected out and a clamp was placed either above the bifurcation or else clamps were placed on the major branches just below the bifurcation An attempt was made to visualize the septal artery and it was ligated whenever possible The chest was closed the pleural space aspirated and the animal given an injection of penicillin in a dose of 300 000 units

In animals later subjected to right coronary arterial occlusion a similar technique was used after a time interval of at least one month An effort was made to place the clamp on the base of the right coronary artery before branches are given off

A group of 6 pigs was also prepared in a similar fashion Most of these animals required tracheotomy for intubation and these animals were maintained on inhalation anesthesia using cyclopropane or ether

Postoperatively, the animals were observed and repeated electrocardiograms were obtained Progress of closure of the clamps was studied with serial x rays Some of the dogs were subjected to coronary arteriography using a catheter introduced through one of the carotid vessels

The surviving animals were sacrificed from 1 to 18 mo after operation At the time of sacrifice careful observation was made of the appearance of the heart presence of infarcts and status of the clamps The heart was injected with a 20 per cent suspension of bismuth oxychloride and sections taken for microscopic examination

## RESULTS

Thirty two dogs were prepared as described above At the time of death or sacrifice 24 animals were found to have total occlusion of one or more major coronary arteries The results for this group are listed in Tables 1 and 2 The group of 8 animals in which the clamps did not close sustained a considerable degree of partial occlusion This was estimated as high as 90 per cent for some of the arteries

*Table 1 Mortality Following Gradual Coronary Arterial Occlusion*

| LOCATION OF OCCLUSION | NUMBER OF DOGS | DEATHS | MORTALITY |
|---|---|---|---|
| Left main coronary artery | 12 | 11 | 92% |
| Left circumflex and anterior descending branches at bifurcation | 3 | 0 | 0 |
| Left circumflex branch | 3 | 1 | 33% |
| Left anterior descending branch | 2 | 1 | 50% |
| Right coronary artery* | 6 | 1 | 17% |

*Five dogs in this group had previous operations on the left coronary artery (See Text)

*Table 2 Results in Dogs Surviving Gradual Coronary Arterial Occlusion*

| OCCLUSION SUSTAINED | NUMBER OF DOGS | INFARCTION |
|---|---|---|
| Left and right main coronary arteries | 1 | 0 |
| Left circumflex and anterior descending branches at bifurcation | 3 | 0 |
| Left circumflex branch and right coronary artery | 1 | 0 |
| Left circumflex branch | 1 | 0 |
| Left anterior descending branch | 1 | 1 |
| Right coronary artery | 3 | 1 |

Of 12 dogs which were prepared with a single clamp applied to the base of the left coronary artery 11 died within a month of operation One animal survived and a month later a second clamp was placed on the base of the right coronary artery Several postoperative electrocardiograms obtained on this animal were interpreted as normal He died 14 months after the second operation following general anesthesia Autopsy showed that the entire left coronary artery including the septal branch was occluded as was the right coronary artery Injection with bismuth oxychloride suspension via the anterior descending branch of the left coronary artery outlined an essentially normal coronary arterial tree No infarcts were present either grossly or microscopically

Three animals were prepared with separate clamps placed around the anterior descending and the circumflex branches of the left coronary artery These animals all survived total occlusion of the left coronary artery Of 3 dogs with a single occluding clamp on the left circumflex branch 2 survived One of 2 dogs with occlusion of the anterior descending branch of the left coronary artery survived

Five out of 6 dogs survived occlusion of the right coronary artery This group included 1 dog with additional occlusion of the left main coronary artery another with occlusion of the circumflex branch of the left coronary

artery and 3 dogs in which the left coronary arterial clamps had failed to close. Of the surviving animals 1 had a 3 cm infarction of the anterior wall of the right ventricle. Arteriograms taken on 2 of the animals in this group showed an extensive collateral circulation developing between the occluded right coronary artery and the patent left coronary artery.

Table 2 shows the results in dogs surviving gradual coronary occlusion. Of the 10 surviving animals only 2 showed gross or microscopic evidence of infarction.

In addition to the group of dogs described above, 6 pigs were studied. Three animals died suddenly 28, 15 and 60 days after operation. Two animals were found to have complete occlusion of the left main coronary artery without infarction and a third pig had occlusion of the anterior descending branch also without infarction. It is presumed that these animals developed ventricular fibrillation at the time of death.

In 2 large pigs partial erosion of the clamp through the vessel wall with subsequent recanalization of the coronary artery was noted.

One animal survived occlusion of the right coronary artery. At the time of sacrifice 9 mon. after operation a 2 by 3 cm. infarct was found on the anterior wall of the right ventricle near the base.

## DISCUSSION

A distinct difference can be noted between the effects of acute and chronic occlusion of the coronary arteries. It appears that when the coronary arterial system of the dog is subjected to gradual obliteration by a suitable method *a significant and useful collateral circulation develops*. This is corroborated by the high survival rate, minimal number of infarcts and the lack of electrocardiographic changes. This difference between acute and chronic occlusion *of the coronary arteries might be due to one or more of the following* factors: 1. Increase in blood flow from venous system to capillary bed; 2. Increase in blood flow in intercoronary collateral circulation; 3. Increase in extracardiac collateral *circulation*; 4. Decrease in incidence of ventricular fibrillation.

Of these, intercoronary anastamoses would appear to be the most likely protective mechanism. Evidence for this is seen in the arteriograms which show large intercoronary anastamoses connecting the coronary artery beyond the point of occlusion to another artery. That there is a limit to the ability of the heart to respond to gradual occlusion is brought out by the fact that 11 out of 12 dogs with a single clamp placed on the base of the left coronary artery died.

Wiggers[6] has pointed out that intercoronary anastamoses are frequently present between the 3 principal coronary arteries in the dog but that their presence in normal human hearts is controversial. In view of this one can doubt the wisdom of using the results of experiments on the dog's heart as the valid basis of making prediction for the course and surgical treatment of human coronary arterial disease.

The lack of naturally occurring intercoronary anastamoses in the porcine heart, as *pointed out by Eckstein*,[7] *would* seem to make this animal more suitable for studies dealing with myocardial revascularization. In addition the anatomic arrangement of the coronary arterial system of the human and pig heart is quite similar. However, continued *growth* even with the Hormel

Institute midget pigs which were used in 4 experiments makes long term study difficult

Erosion of the present style clamp through the large coronary arteries of the pig was disturbing and a slightly different style clamp is being investigated in the hope of obviating this problem

*SUMMARY*

Using a slowly closing stainless steel clamp whose jaws are initially separated by surgical gut a group of dogs and pigs were subjected to chronic coronary arterial occlusion The canine heart can withstand a high degree of occlusion if this is carried out in a gradual fashion over a period of about one month

Evidence seems to point to intercoronary anastamoses as the source of blood for the occluded areas

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# DETERMINATION OF MYOCARDIAL BLOOD FLOW UTILIZING IODINATED ($I^{131}$) HUMAN SERUM ALBUMIN*

WALTER S HENLY OSCAR CREECH JR CECIL M COUVES MARY C MORGAN DON W CHAPMAN AND HERBERT C ALLEN, JR

The lack of a satisfactory method of determining myocardial blood flow in the intact animal has limited investigation of medical treatment of coronary artery disease and of surgical procedures designed to augment the coronary circulation This report is concerned with a new method of determining myocardial blood flow in the intact animal The method is based upon the rate of return of radio iodinated ($I^{131}$) human serum albumin to the right

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heart after injection of a known quantity of RISA into the left heart. In the preliminary investigation RISA was injected through a thoracotomy incision directly into the left ventricle of the dog while taking a continuous sample of blood from the right ventricular outflow tract. Later experiments were performed during cardiac catheterization in the intact subject.

METHOD

Cournand cardiac catheters (#8) are placed via the external jugular vein into the pulmonary wedge and the right ventricular outflow tract under fluoroscopic control. The blood pressure and the ECG are recorded during the determination. Cardiac output is determined by the direct Fick principle immediately preceding and following the myocardial blood flow determination. Polyethylene tubing (PE 205) 30 ft. long filled with heparinized physiologic saline solution is connected to the catheter in the right ventricular outflow tract, and withdrawal of blood from the heart at a constant rate for 60 sec. is begun. A known quantity (200 to 750 microcuries) of RISA is injected into the pulmonary wedge in 1 sec. using an automatic injector. An immediate radiodilution curve is obtained by monitoring the tubing with a scintillation counter and recording with an Esterline Angus Graphic Meter. Immediately following the procedure the polyethylene tubing is rescanned for a more accurate quantitative determination. The residual radioactivity in the injection system is determined and the corrected quantity of RISA injected into the pulmonary wedge is calculated.

RESULTS

During the first minute following injection, the changing concentration of RISA in the right ventricle could be determined giving a composite radiodilution curve of a typical pattern (Fig. 1). By simultaneous sampling from the right ventricle, coronary sinus, superior vena cava and inferior vena cava, the components of this dilution curve were apparent (Fig. 2). In all animals the superior vena caval return did not occur until more than 5 sec. following the appearance of RISA in the right ventricle. Thus the radioactivity during the first 5 sec. of the dilution curve is contributed by the myocardial circulation and its rate of appearance in the right heart is a measure of myocardial blood flow. Ligation of the left main coronary artery significantly reduced the appearance rate. Immediate ligation of the coronary sinus produced no significant alteration in the rate of flow. The reproducibility of the method was demonstrated in 20 dogs using the open chest technique. Similar radiodilution curves could be obtained by injection of RISA into the pulmonary wedge in the intact animal.

To quantitate the myocardial blood flow in cc. per minute, 2 separate dilutions must be considered. The injected RISA is mixed first in the left heart. The dilution from the pulmonary wedge to the left atrium is insignificant. In the dog the injected volume has been large in comparison to the stroke volume and the dilution of RISA occurs over a 5 sec. period. During this time the RISA is diluted by a volume of blood equal to the cardiac output during the dilution period (c.o./sec. × t sec.). In the adult human the volume of the injected dose is small in comparison to the stroke volume and mixing is assumed to occur with a single beat of the heart. Hence a volume of blood of definite concentration of RISA is ejected from the left ventricle

NORMAL DOG RADIODILUTION CURVE

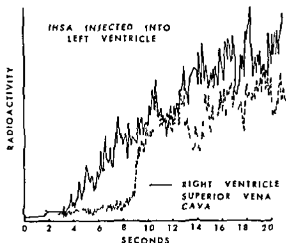



Fig 1

RADIODILUTION CURVE COMPONENTS
17 KG DOG

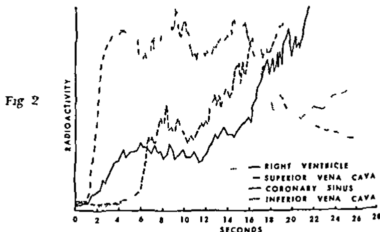



Fig 2

A second dilution is constantly occurring on the right side of the heart This is the same regardless of the size of the animal and is equal to the volume of blood passing through the right ventricle during the sampling period At any instant the quantity of RISA in the right heart will represent a definite volume of coronary flow From the previous concepts the following formulae were derived in order to determine quantitatively myocardial blood flow

In the human

$$MBF = \frac{(c.o./sec)\ (stroke\ volume)\ (avg\ conc\ RISA\ )_{t\ rv}\ (60)}{RISA_{pw}}$$

In the dog

$$MBF = \frac{(c.o./sec)^2\ (avg\ conc\ RISA_{rv})_t\ \ (300)}{(RISA)_{pw}}$$

Where $RISA_{pw}$ = microcuries of RISA injected into the pulmonary wedge

RISA = microcuries of RISA in the right ventricle

*C o /sec = cardiac output/second*

t = 5 seconds

MBF = Myocardial Blood Flow in cc /minute

*Table 1 Myocardial Blood Flow—Animal Data*

| B P | MBF | % C O |
|---|---|---|
| 130/110 | 101 cc | 5.7% |
| 115/100 | 28.5 cc | 5.4% |
| 130/115 | 183 cc | 8.6% |
| 50/40 | 21.5 cc | 3.3% |
| 140/100 | 100 cc | 5.6% |

*Table 2 Myocardial Blood Flow—Human Data*

| | MBF | % C O |
|---|---|---|
| Normal ECG | | |
| WM 33 | 316 cc/min | 4.0% |
| CM 57 | 311 | 6.1% |
| CM 30 | 205 | 8.0% |
| Abnormal ECG | | |
| CM 59 | 123 cc/min | 2.8% |
| WM 61 | 171 | 5.1% |
| WM 62 | 163 | 4.4% |

*Table 3 Myocardial Blood Flow Calculated vs Measured Flow*

| B P | MBF (CALCULATED) | B P | MBF (MEASURED) |
|---|---|---|---|
| 130/115 | 131 cc/min | 105/95 | 98.3 cc/min |
| 140/100 | 100 | 60/50 | 73 |
| 130/110 | 70.7 | 70/55 | 45 |

The results obtained from preliminary determinations in dogs and humans are in the predicted range of myocardial flow and support the original assumptions concerning the manner of dilution of the RISA (Tables 1 and 2). To compare the calculated myocardial blood flow with a direct measurement of coronary sinus and thebesian flow into the right ventricle, extracorporeal circulation was instituted with a pump oxygenator, the pulmonary artery was occluded and venous return to the right ventricle was collected and measured. The results correlated well with those obtained using RISA (Table 3).

## SUMMARY

In summary, a new method of determining myocardial blood flow utilizing iodinated ($I^{131}$) human serum albumin has been described. It is applicable to the intact individual during routine cardiac catheterization with an adequate margin of safety. With additional refinements in technique the method should prove useful in evaluating problems associated with coronary artery disease.

# A PRELIMINARY REPORT ON A RADIOISOTOPIC METHOD FOR MEASURING CEREBRAL BLOOD FLOW*

PAUL H CRANDALL AND BENEDICT CASSEN

The need for a method of study for portions of the cerebral circulatory system is, we believe, fulfilled by the radioisotopic angiogram proposed here. At present the principal methods for estimating cerebral circulation are the appearance of the contrast angiogram and the nitrous oxide method. The nitrous oxide technique yields a quantitative over-all value of blood flow quoted for the normal young adult as 51 cc ($\pm$12)/100 gm brain/min.[1] The contrast angiogram gives a serial radiographic record after the intra-arterial injection of various contrast media but does not lend itself to quantitative measurement of blood flow. The method proposed here has been used as a supplement to the contrast angiogram of the carotid torcular circulation for the specific purpose of determining cerebral blood flow and circulation time.

## METHOD

All patients chosen for this study were undergoing diagnostic carotid angiograms for various clinical reasons. The agent used was radioactive iodinated ($I^{131}$) serum albumin (RISA), the safety of which has been well attested. The amounts used were well below any hazardous level.

A marker was placed on the patient's scalp either on the inion or at the junction of the external occipital crests with the sagittal suture. Using local anesthesia a #19 or #20 gauge spinal needle was inserted percutaneously into a common carotid artery. A seriographic record was then made following intra-arterial injection of 8 cc. to 10 cc. of Hypaque. These pictures were used for qualitative correlation with the subsequent radioactive angiogram and, also to determine accurately the position of the torcular Herophili in relation to the external scalp marker.

Following the contrast study a radioactive blood flow study was made. The equipment consisted of a well collimated, shielded scintillation counter that recorded onto a scintiscaler and Sanborn Visocardiette mounted as a portable unit with the counter on a movable arm so that the entire unit may be adapted to many locations and positions (Fig. 1). The counter aperture is 1 in. in diameter. With the patient's head in a lateral position the counter aperture was placed directly over the torcular as determined by measurements derived from the previous study and scalp marker. In addition using a simple goniometer one arm of which was on a line between the lateral limbus of the eye and the external auditory meatus the counter was then placed at an angle of 30° with a plane parallel to the Frankfort plane.

After a background activity of 10 sec. or more was determined a dose of 10 to 20 microcuries of RISA was injected in the common carotid. The variations in activity were recorded in counts on the Sanborn Visocardiette for 16 to 18 sec. After an equilibrium was reached (5 min. plus) this value was recorded for 10 or more sec. and a simultaneous blood sample from a suitable vein was drawn to determine blood volume. The dilution curve was then plotted in terms of counts per 2 sec.

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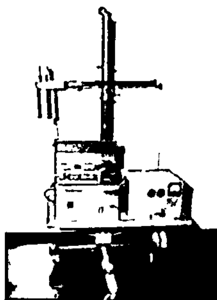


Fig 1 Recording unit consisting of a scintillation counter scintiscaler and Sanborn Visocardiette

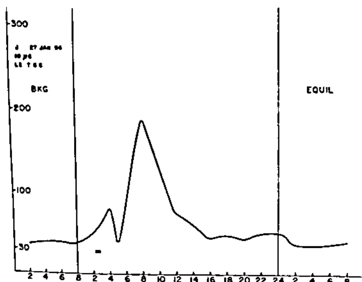



Fig 2 Dilution curve over torcular Herophili in a normal young adult

Figure 2 represents the usual response in a normal young individual It will be seen that the large curve does not return to the level of the background activity and hence the remainder of the dilution curve is assumed to be a simple exponential and is therefore extrapolated The difference which is due to the effect of circulation other than that in the torcular is so small that minimal error is possible by this extrapolation Using an equation

$$\text{Cerebral flow} = \frac{\text{Equilibrium}}{\text{Total count of curve}} \times \text{total blood volume} \times 60$$

the cerebral blood flow through the torcular is obtained

The size of the dose of RISA is important only in obtaining a curve that is as high as possible without exceeding the counting ability of the Sanborn Visocardiette the higher the curve the more accurate the determination We have found it useful to use 20 microcuries in 2 cc of RISA as the usual dose to be administered

## DISCUSSION

The scintillation counter in effect subtends a beam the angle of which is 26° When placed in the specified anatomical position, it sees the following large blood vessels the torcular Herophili the vertebral artery and basilar venous plexus and the carotid arteries and cervical veins Considering the RISA as a bolus of material passing through the system immediately after injection the carotid arteries the torcular and the cervical veins are seen in sequence but sufficiently spaced in time so that the 3 components can be measured

Corroboration of this effect was noted in examinations of 2 patients who had thrombotic occlusion of an internal carotid artery When the RISA was injected in the common carotid below the site of thrombosis an initial rise during the first second occurred but was followed by a fall to the pre existing background level a second rise, 12 sec after injection represented a cervical venous flow from the external carotid circulation The main curve ordinarily contributed by the torcular was absent In the majority of patients we have been able to distinguish these 3 portions of the curve

The effect of distance must be evaluated In considering a point source in front of the counter, the value for radiation falls off as the inverse of the square of the distance so that in this situation the layer of RISA nearest the counter contributes by far the largest part of the radiation curve Since in the injection phase the RISA is passing as a bolus through the system and since the torcular is such a large vascular structure closely applied to the skull and near to the counter, the main dilution curve can be attributed to torcular flow The accuracy for determination of blood flow in the peripheral circulatory system depends upon the relative isolation of the structure being measured In the equilibrium phase however this situation is altered The dose of RISA has become diluted in the total blood volume and is homogeneously mixed in all the vascular structures seen by the counter Phantom models of the torcular Herophili are currently being used to determine what fraction the torcular count contributes to the measured equilibrium value It is expected that the results of the phantom study will enable the blood flow to be stated in absolute cc /min for comparison with nitrous oxide results Until a number of these phantoms are tested the results indicate only relative values For the derivation of the mathematical formula used in calculating blood flow we refer to the article by MacIntyre Pritchard Eckstein and Friedell[2] for the determination of cardiac output Others have also reported successful determination of cardiac output by these methods[3]

## RESULTS

Determinations by the radioisotopic method for carotid torcular blood flow were made on 16 patients On only the last 8 however was the technique sufficiently standardized for quantitative measurements

1 Circulation time was measured from the beginning of injection to the highest point of the torcular phase Among 5 young adults this measured 5 0 to 6 2 sec the average was 5 6 sec The remaining 11 patients had clinical diagnoses of brain tumor (3) cerebral arteriosclerosis (4) subarachnoid hemorrhage (1) internal carotid thrombosis (1) occlusion of arterior choroi

dal artery (1) and traumatic absence of frontal lobe (1) Among these 11 patients the circulation time varied between 5 1 and 8 3 sec The latter occurred in a patient with marked cerebral arteriosclerosis

2 Characteristics of the dilution curve were analyzed All patients curves showed an initial small rise during the first 2 sec attributable to carotid blood flow The succeeding elevated curve represented torcular blood flow In 5 normal young adults this curve occurred between the second second and the twelfth to fourteenth seconds Among the abnormalities noted in this phase the patient with the prolonged circulation time also exhibited a prolongation of the torcular phase through the eighteenth second both observations were significant in the face of cerebral arteriosclerosis One patient with subarachnoid hemorrhage had a prolonged torcular phase through the sixteenth second One patient with a pineal tumor showed a doubly peaked dilution curve during the torcular phase which in this instance we believe is a manifestation of delayed circulation through the internal cerebral veins and central regions

3 Quantitative blood flow through the torcular Herophili was calculated in relative and actual values For purposes of accuracy the elevation of the torcular phase must be near to or exceed 300 counts/sec Among 5 normal young adults under these conditions the relative values were 210 to 310 E cc./min As mentioned previously the equilibrium value is currently being studied and hence this unknown E has been left in the relative value

## SUMMARY AND CONCLUSIONS

A radioisotopic method with external counting technique for the determination of blood flow through the torcular Herophili is presented Radioactive iodinated ($I^{131}$) serum albumin is injected into a common carotid artery and the blood flow recorded by a scintillation counter placed at a specified angle over the torcular Herophili The theory and procedure of the test are explained The characteristics of the dilution curve the carotid torcular circulation time and the relative volumetric results of blood flow are reported in a small group of patients including normal young adults and others with various clinical conditions Further plans include determination of absolute values and accuracy in a larger number of tests

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group studied comprised 58 patients selected on the same grounds as for the renal studies

RESULTS AND DISCUSSION

**Renal Function Studies** Because of its proven accuracy one is well justified to use the creatinine clearance test[2, 3, 4] as the starting point of the assessment of the findings The results of this test have a wide range of normal variation given by most authors as 70 to 130 ml /min or 100 ml ± 30 per cent per min In this study only downward variations of 10 per cent or more were regarded as significant (for the sake of brevity the detailed discussion of the reasons for this will be omitted) Nine out of the 50 cases studied were found to show a reduction of function of this magnitude or greater (Table I) It must be noted however that in 3 of these 9 cases although the drop was 15 per cent the post injection values were still considered to be low normal i e above 70 ml /min All of these cases on the other hand exhibited a distinct and significant concomitant rise in the serum creatinine and 2 of the cases also showed a significant decline in phenolsulfonphthalein excretion

The precise nature of the insult inflicted upon the kidney by the injection of Urokon leading to functional impairment is not known but it is generally assumed to be a chemical trauma This assumption is supported by the fact that of the 9 cases showing significant changes 6 were examples of occlusive disease in which the impaired outflow allows a higher concentration of the contrast medium to come into contact for a longer period of time with the renal parenchyma than is the case in aneurysms or after the obstruction is removed by grafting Conversely only 2 out of 17 cases with patent graft and only 1 out of 10 aneurysms showed impairment of function after the injection

The onset of the damage caused by the contrast dye appeared to be instantaneous and its duration short as a rule In most instances the renal function returned to normal within 24 to 48 hrs However in 1 case normal renal function was not restored for 11 days after which a successful operation could be performed with uncomplicated recovery (Case 51) The correspondence of the values of serum creatinine with the results of creatinine clearance was quite close both in the cases listed in Table I showing functional post aortographic impairment and in almost all of the other cases Thus the serial determination of serum creatinine levels can be used as a simple and accurate means of following the changes in renal function after aortography

**Cardiac Studies** The most common symptom after aortography that can be related to the heart was chest pain which was present in about 10 per cent of the patients on whom we performed this examination without general anesthesia Although this pain is often indistinguishable from angina pectoris apparently in most instances it is not caused by coronary spasm and myocardial ischemia but rather by the flooding by the dye and consequent constriction of the intercostal arteries The incidence of this symptom among the patients showing electrocardiographic changes was no higher than among the other cases comprising the group

Pulse and blood pressure changes occurred commonly but without a definite trend and the changes showed no correlation with electrocardiographic alterations

# RENAL AND CARDIAC RESPONSES AFTER TRANSLUMBAR AORTOGRAPHY*

D EMERICK SZILAGYI ROGER F SMITH CLAIBOURNE P SHONNARD AND HERNAN ALVAREZ

With the current widespread use of translumbar aortography the physiological changes brought about by the introduction of contrast media into the abdominal aorta have assumed the importance of a practical clinical problem Instances of significant renal and cardiac functional aberrations observed after aortography and the lack of sufficient basic information with respect to the physiologic responses to the intra aortic injection of radio opaque substances have prompted us to investigate two aspects of this problem

## METHODS

**Technique of Aortography** The well standardized technique described by Smith *et al*[1] was employed with some slight modifications Twenty five to 35 ml of 70 per cent Urokon was injected manually in 1 to 6 sec If it was necessary and safe the injection was repeated once or twice

**Renal Function Studies** For the investigation of the pre and post injection state of the renal function the 24 hr creatinine clearance[2 3 4] and the 15 min phenolsufonphthalein excretion tests were used Main reliance was on the creatinine clearance test These tests were chosen for the following practical reasons They are relatively simple to carry out and yet they have well documented accuracy and reliability moreover they afford an estimate of both glomerular (creatinine clearance) and tubular (phenolsufonphthalein excretion) function The method of creatinine determination was that of Bonsnes and Taussky[2] The pre injection study was performed on the day preceding the aortogram and the post injection study on the day immediately following In some cases the clearance was repeated and the serum creatinine was followed seriatim The group studied comprised 50 patients of varying clinical background so selected as to provide a representative sample of the class of patients likely to be submitted to aortography

**Cardiac Studies** Electrocardiograms were taken the day preceding the aortogram With the patient in the prone position a preliminary electrocardiogram was obtained just before the injection of the contrast medium Electrocardiographic tracings were then made immediately after the injection of the dye and five 10 and 15 minutes later Records were kept of the pulse rate and blood pressure taken at 5 min intervals The anesthesia was deliberately varied according to the following scheme pentothal sodium (25 cases) intraspinal novocaine (18 cases) intraspinal novocaine with nitroglycerine premedication (15 cases) In the last named group 0 6 mg of nitroglycerine was administered sublingually 3 min before the start of the injection The pre anesthetic medication was uniform for all groups and consisted of morphine atropine and barbiturates The electrocardiographic tracings were obtained through 3 standard limb leads and 3 augmented unipolar leads The recording apparatus was a Cambridge string galvanometer The

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*Table 2 Electrocardiographic Changes After Translumbar Aortography in 16 Cases Among 59 Studied*

| Case No. Age Sex | Type of Vascular Disease | Pre-existing Cardiac Disease | Type of Anesthesia | Electrocardiographic Changes: Before Injection | Electrocardiographic Changes: After Injection |
|---|---|---|---|---|---|
| 5 64 M | Dissecting Aneur | ASHD with Angina | Pentothal Sodium | ST depressed in II and III | ST flat in II and III |
| 9 59 M | Leriche incomplete | ASHD | Spinal | T wave normal<br>T wave normal | T wave tall in II<br>T wave inverted in AVL |
| 13 57 M | Occluded LCI graft | 0 | Spinal | T wave normal<br>T wave normal | T wave tall in II<br>T wave tall in II |
| 15 48 M | Leriche | 0 | Pentothal Sodium | NSR<br>ST normal T wave normal | NSR with nodal premature beats<br>ST depressed in II T wave flat in II |
| 18 59 M | Leriche | 0 | Pentothal Sodium | ST normal | ST depressed in II and III |
| 19 59 M | LEI graft REI graft | 0 | Pentothal Sodium | T wave normal | T wave flat in II & AVL, inverted in III |
| 22 67 F | REI occlusion | ASHD with hypertension | Pentothal Sodium | LBBB complete | LBBB with PVS |
| 24 55 M | Bil CI Aneur | ASHD | Pentothal Sodium | T wave small in I flat in AVL | T wave flat in I inverted in AVL |
| 32 68 F | Diffuse AS | ASHD with failure | Pentothal Sodium | AF<br>T wave diphasic in AVF | AF with PVS<br>T wave inverted in AVF |
| 36 67 M | Bil SF Occlusion | 0 | Spinal | NSR QRS 0 07<br>T wave normal | NSR with nodal premature beats QRS 0 12 T wave inverted and coved in I and III |
| 43 50 M | RCI Occlusion | 0 | Spinal with premed | T wave normal | T wave inverted in II and III |
| 44 62 M | Abd Aortic Aneur | ASHD | Spinal | NSR ST normal<br>T wave normal | NSR with PVS ST depressed in I<br>T wave inverted in II and III |
| 53 59 M | LCI and LSF Occlusion | ASHD | Spinal with premed | NSR | NSR with nodal premature beats |
| 57 51 M | RSF Graft | 0 | Pentothal Sodium | NSR | NSR with PVS |
| 58 55 M | Leriche | ASHD with Coronary Occl | Spinal | NSR T wave flat in II slightly inverted in III and AVF | Ventricular bigeminy T wave diphasic in II inverted and coved in III and AVF |
| 61 52 M | RCI Occlusion | 0 | Spinal with premed | NSR | NSR with PVS interpolated |

*Abbreviations* L left R right CI common iliac EI external iliac SF superficial femoral AS arteriosclerosis ASHD arteriosclerotic heart disease NSR normal sinus rhythm LBBB left bundle branch block AF auricular fibrillation PVS premature ventricular systole

*Table 1 Changes in Renal Function After Translumbar Aortography in 9 Cases Among 50 Studied*

| CASE NO AGE SEX | SYSTEMIC AS | TYPE OF VASCULAR DISEASE | AMT OF DYE INJECTED | BUN | URINALYSIS PRE ALB | URINALYSIS PRE SED | URINALYSIS POST ALB | URINALYSIS POST SED | CREATININE CLEARANCE ml/min PRE | CREATININE CLEARANCE ml/min POST | PER CENT DROP | SERUM CREATININE mg% PRE | SERUM CREATININE mg% POST | 15 MIN PSP EXCRETION PRE | 15 MIN PSP EXCRETION POST |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 48 M | 1+ | AI Occl Dis | 70 ml | 15 | 0 | 0 | NI | NI | 146 | 65 | 65% | 8 | 1.7 | 35% | 12% |
| 4 51 M | 1+ | PO gr Fem Occl Dis | 90 ml | 18 | 0 | 0 | + | RBC | 156 | 62 | 60% | 8 | 1.9 | 35% | 20% |
| 17 63 M | 2+ | IO gr AI Occl Dis | 40 ml | 15 | 0 | 0 | Tr | 0 | 130 | 75 | 45% | 6 | 1.3 | 37% | 41% |
| 32 60 M | 3+ | I Occl Dis | 90 ml | 13 | 0 | 0 | Tr | RBC | 191 | 107 | 45% | 7 | 1.3 | 4% | 6% |
| 36 65 F | 3+ | I Occl Dis | 105 ml | 14 | 0 | 0 | Tr | RBC | 98 | 23 | 75% | 6 | 2.1 | 28% | 12% |
| 38 39 M | 1+ | AI Occl Dis | 70 ml | 17 | 0 | 0 | Tr | RBC | 168 | 42 | 75% | 6 | 2.1 | 26% | NI |
| 45 56 M | 3+ | IO gr Abd Aneur | 105 ml | 12 | 0 | 0 | 0 | 0 | 187 | 104 | 45% | [illegible] | 1.0 | 24% | 16% |
| 47 48 M | 2+ | AI Occl Dis | 40 ml | 11 | 0 | 0 | 0 | 0 | 116 | 54 | 55% | 1.0 | 1.3 | 16% | 0% |
| 51 41 F | 2+ | AI Occl Dis | 80 ml | 9 | 0 | 0 | +++ | RBC | 122 | 33 | 75% | 6 | 2.3 | 34% | 6% |

*Abbreviations* AI aorto iliac I iliac gr graft Pre pre aortogram Post post aortogram NI no information AS arteriosclerosis

3. Camara, A. A., *et al.* The twenty-four hourly endogenous creatinine clearance as a clinical measure of the functional state of the kidneys. J. Laborat. Clin. M. 37: 743, 1951.
4. Haugen, H. N., and Blegen, E. M. Plasma creatinine concentration and creatinine clearance in clinical work. Ann. Int. Med. 43: 31, 19

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# HYPERHYDRATION: A METHOD OF DECREASING THE NEPHROTOXIC EFFECTS OF UROKON AS EMPLOYED IN AORTOGRAPHY*

GEORGE C. MORRIS, JR., E. STANLEY CRAWFORD, ARTHUR C. BEALL, JR., AND JOHN H. MOYER

Renal insufficiency following translumbar aortography has been encountered with increasing frequency during recent years.[1,2,3] A clinical impression was gained by the authors that patients who were given 1 or 2 liters of intravenous fluid immediately prior to aortography did not develop evidence of renal damage. Conversely, patients in the usual pre-anesthetic and semi-dehydrated state frequently demonstrate minimal and occasionally marked elevation of the blood urea nitrogen following aortography. The necessity for a satisfactory urinary output in the prophylaxis of renal injury and crystalluria during treatment with certain sulfa derivatives is well accepted. Less well documented is the importance of dehydration in the development of renal insufficiency associated with hemoglobinuria.[4,5] With this background 2 groups of dogs were studied to determine the effects of hyperhydration and relative dehydration at the time of intra-aortic injection of nephrotoxic doses of 70 per cent Urokon (R) sodium.

## METHOD

Mongrel female dogs were anesthetized with intravenous pentobarbital (30 mg./kg.) after hydrating with water through a gastric tube (40 cc./kg.). Control determinations of renal function were then made using averages of three 10 min. collection periods. Creatinine was used to measure glomerular filtration rate (GFR). Renal blood flow (RBF) was derived from renal plasma flow (RPF) measured by para-aminohippurate. The methods and techniques have been described previously.[6] Later, the abdominal aorta was exposed through a retroperitoneal left flank incision. The aorta was occluded below the origin of both renal arteries and 70 per cent Urokon (R) sodium in a dose of 6 cc./kg. was injected rapidly through a #18 gauge needle into the aorta above the occluding clamp (Fig. 1). After removing the aortic clamp, the wound was sutured in layers and the dog given intramuscular

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*From the Cora and Webb Mading Department of Surgery and the Department of Pharmacology, Baylor University College of Medicine, Houston, Texas. Supported in part by grant H 1847 from the National Heart Institute of the National Institutes of Health, United States Public Health Service, and a research grant from the Houston Heart Association.

Among the 58 cases studied, 16 showed electrocardiographic post-injection changes that were judged significant (Table 2). The most common finding was inversion of the T wave (in 9 cases) and depression of the ST segment (3 cases). In 6 cases arrhythmia was noted. These comprised mainly premature nodal and premature ventricular systoles. All the changes were instantaneous or at least followed closely the injection of the dye. In 1 instance (Case 5) the depression of ST segment developed gradually over several hours. The alterations were generally transient, but in the last named case they persisted for 3 days.

The exact mechanism of the effects of the injection of dye on the heart is not clear. On the whole, the electrocardiographic changes can readily be explained by myocardial ischemia, most probably resulting from coronary spasm. The obvious mode of production of this spasm would be the irritant action of the dye on the intima of the coronary arteries. There is evidence among our angiograms that the dye injected into the abdominal aorta at the level of the twelfth thoracic or first lumbar vertebra may reflux into the thoracic aorta and indeed into the aortic arch. Thus coronary spasm consequent on intimal irritation by the contrast medium must be regarded as the most probable mechanism underlying the electrocardiographic changes. The reflex systemic arterial constriction seen at times following intra-arterial dye injection may add to this response, and the possibility of some noxious effect directly on the myocardium cannot be excluded.

A question of much practical importance is whether pre-existing heart disease and the presence of severe distal occlusive disease increase the likelihood of the occurrence of the changes described above. The data at hand are insufficient to justify definite conclusions, but they suggest that the answer is in the affirmative.

## SUMMARY AND CONCLUSIONS

Before and after the translumbar intra-aortic injection of 70 per cent Urokon in 50 patients the changes in renal function were followed by means of 24 hr. creatinine clearance, 15 min. phenolsulfonpthalein excretion, and serial serum creatinine determinations, and in 58 patients the alterations in pulse, blood pressure and electrocardiographic tracings were recorded. In 18 per cent of the patients in the first group significant reduction of the renal function was noted. Twenty-eight per cent of the patients in the latter group showed electrocardiographic changes, mostly those indicative of myocardial ischemia. Both types of functional aberration were instantaneous and virtually always transient, as a rule clearing in 48 hrs. In selecting patients for aortography and in timing surgical interventions after aortography, the possibility of these physiologic reactions must be kept in mind. Checkup electrocardiographic examinations and serial serum creatinine determinations before and after aortography appear to be obligatory precautionary measures.

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4 Hauren H N and Blegen F M Plasma creatinine concentration and creatinine clearance in clinical work Ann Int Med *43* 731 19

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## METHOD

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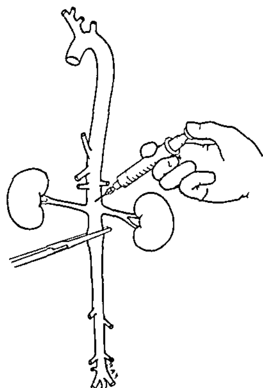


Fig 1 The method of intra aortic injection of Urokon (R) A vascular clamp occludes the aorta distal to the renal arteries to potentiate the quantity of Urokon entering the renal arteries A retroperitoneal left flank incision offers satisfactory exposure

penicillin The purpose of aortic occlusion distal to the renal arteries during the injection of Urokon (R) was to promote maximal renal damage from a nonlethal quantity of the drug Three to 5 days later renal function studies were repeated Alternate dogs in the series were prepared before intra aortic injection with the following 2 techniques in 1 group water was withheld for 24 hrs just prior to operation Dogs in the second group were allowed unrestricted water intake and then given 40 cc /kg of 5 per cent glucose in water intravenously immediately prior to the intra aortic injection After operation both groups were allowed unrestricted water intake On completion of the experiment, the animals were sacrificed and the kidneys sectioned for gross and microscopic changes

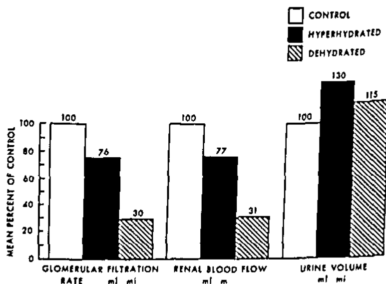



Fig 2 A comparison of the glomerular filtration rate renal blood flow and water excretion following intra aortic injection of Urokon (R) Renal functional impairment is significantly greater in the animals that were dehydrated at the time of injection

*Table I Comparison of Renal Functional Impairment Following Intra aortic Injection of Urokon(R) in the Dehydrated State Compared with the Hyperhydrated State*

| DOG | Dehydrated | HEMATOCRIT | | GLOMERULAR FILTRATION RATE ml/min | | RENAL BLOOD FLOW ml/min | |
|---|---|---|---|---|---|---|---|
| | | C | U | C | U | C | U |
| 1 | | 44 | 47 | 78 | [illegible] | 357 | 66 |
| 3 | | 43 | 43 | 47 | 30 | 337 | 195 |
| 5 | | 36 | 34 | 58 | 32 | 344 | 223 |
| 7 | | 50 | 39 | 86 | 8 | 530 | 53 |
| 9 | | 35 | 31 | 42 | 7 | 208 | 20 |
| 11 | | 42 | 27 | 61 | 6 | 505 | 15 |
| 13 | | 40 | 42 | 34 | 8 | 210 | 45 |
| 15 | | 25 | 26 | 36 | 18 | 136 | 86 |
| 17 | | 43 | 38 | 46 | 42 | 237 | 213 |
| 19 | | 35 | 28 | 37 | 2 | 228 | 35 |
| 21 | | 43 | 23 | 28 | 3 | 147 | 6 |
| 23 | | 39 | 28 | 49 | 8 | 277 | 36 |
| | Mean | 40 | 34 | 49 | 14 | 293 | 83 |
| | Per cent of control | | 86 | | 30 | | 31 |
| | Hyperhydrated | | | | | | |
| 2 | | 45 | 42 | 63 | 62 | 375 | 338 |
| 4 | | 39 | 39 | 43 | 38 | 179 | 177 |
| 6 | | 40 | 35 | 40 | 43 | 177 | 240 |
| 8 | | 42 | 30 | 38 | 31 | 195 | 166 |
| 10 | | 33 | 29 | 52 | 47 | 264 | 238 |
| 12 | | 26 | 24 | 49 | 23 | 257 | 155 |
| 14 | | 39 | 30 | 43 | 57 | 208 | 240 |
| 16 | | 45 | 36 | 38 | 27 | 244 | 161 |
| 18 | | 39 | 39 | 53 | 51 | 310 | 267 |
| 20 | | 39 | 34 | 35 | 7 | 205 | 55 |
| 22 | | 36 | 36 | 45 | 12 | 203 | 44 |
| 24 | | 31 | 29 | 42 | 17 | 226 | 130 |
| | Mean | 38 | 34 | 45 | 35 | 237 | 183 |
| | Per cent of control | | 89 | | 76 | | 77 |

C – Preoperative control
U – 3 to 5 days after injection of Urokon (R)

## RESULTS

The results are summarized in Table I There was a mean reduction in hematocrit due to sampling and the operative procedure of 14 per cent in the dehydrated group and 11 per cent in the hyperhydrated group Four dogs in the dehydrated group died before the final renal function studies could be made and are not included in the table None of the well hydrated animals died There was a mean reduction in glomerular filtration rate of 70 per cent in the dehydrated group compared to only 24 per cent in the hyperhydrated group Similarly renal blood flow was reduced 69 per cent in the former and only 23 per cent in the latter (Fig 2) A severe depression in renal function as represented by a reduction of more than 70 per cent in

both functions was present in 8 of the dehydrated group and in only 2 of the hyperhydrated dogs. There was no significant difference in blood pressure or urine volume between the 2 groups. Grossly, the kidneys of the dehydrated group showed edema and variable diffuse hemorrhagic changes. These changes were minimal in the well hydrated group. Microscopically, *in addition to edema and hemorrhage there were many tubules filled with* eosin staining material in the dehydrated group. In the hyperhydrated group these changes were minimal or absent.

## DISCUSSION

General or spinal anesthesia is commonly employed clinically during aortography, usually necessitates withholding oral intake for 10 to 18 hrs. Further, because of the brevity of the procedure, intravenous fluids are frequently given sparingly or not at all. Thus a hydropenic state is reached at the time of maximal concentration of the radio opaque medium in the kidney. This state of relative dehydration with associated diminished water excretion should offer both an increased concentration and exposure time of the substance in the nephron. Diuresis should reduce these factors.

In this study the dehydrated group of animals showed severe functional damage while the well hydrated group usually had only minimal damage. The study does not suggest the relative importance of dehydration in promoting or hyperhydration in preventing renal damage. However the rate of water excretion was the common denominator for both groups. Hence the magnitude of damage in any given state of hydration may be related to this function.

## SUMMARY AND CONCLUSIONS

The nephrotoxic propensity of 70 per cent Urokon as used in translumbar aortography was demonstrated in dogs. Functional renal damage was greatest when water was withheld for 24 hrs. In dogs hydrated sufficiently to produce diuresis minimal damage was usually demonstrated. It is reasonable to assume that diuresis reduced both the concentration and the length of the exposure of this nephrotoxic agent in the kidney.

It is suggested that adequate hydration just prior to aortography may help prevent tragic incidents of renal insufficiency.

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# THE VARIOUS EFFECTS OF ANESTHESIA, HYPOTHERMIA, AND RENAL ISCHEMIA ON THE KIDNEY IN PATIENTS HAVING EXCISIONAL THERAPY OF THORACIC ANEURYSMS*

ARTHUR C. BEALL, JR., GEORGE C. MORRIS, JR., MICHAEL E. DEBAKEY AND JOHN H. MOYER

Surgical excision has now been established as the method of choice in the treatment of aneurysms of the aorta. Because hypothermia has been found to provide a protective effect against ischemic damage to the spinal cord,[1, 2] it has been frequently employed in the excision of certain types of aneurysm of the thoracic aorta.[3] The purpose of this study was to determine the various effects on the kidney of the combination of anesthesia, hypothermia and temporary aortic occlusion.

## METHOD

Eleven male and 3 female patients ranging in age from 41 to 61 years were studied. All had fusiform aneurysms involving the upper segment of the descending thoracic aorta and were treated by resection with homograft replacement. Observations were made of mean blood pressure, glomerular filtration rate, renal blood flow, and water and electrolyte excretion. Mean blood pressure was derived from auscultory systolic and diastolic pressures by adding one third of the pulse pressure to the diastolic pressure. Glomerular filtration rate and renal blood flow were determined by the use of inulin and para aminohippurate. Sodium and potassium determinations were made using a Beckman flame photometer. Methods and techniques have been described previously.[4-6] Three 10 min. collection periods were obtained before the patients were anesthetized and two 10 min. collection periods immediately after anesthetization. Hypothermia was then induced using an electrically controlled, water-cooled blanket. Maximum reduction in temperature ranged from 29° to 33° C. Observations consisting of two 10 min. collection periods were made at variable periods somewhat above maximum hypothermia and at the point of maximum reduction in temperature. These were repeated after blood pressure had become stabilized following release of aortic occlusion and again after rewarming. Finally, three 10 min. collection periods were obtained 7 to 14 days after operation. All observations were then subjected to statistical analysis and compared with control values.† Two patients were excluded from the tables as explained below.

## RESULTS

The data obtained from these observations on the effects of anesthesia, hypothermia, and aortic occlusion on renal hemodynamics and water and electrolyte excretion are presented in Table 1. These are represented diagrammatically in Figure 1, which reveal that mean blood pressure was reduced slightly during anesthesia, hypothermia, and following the period of aortic occlusion. These changes, however, are not significant. With the induction of anesthesia there was minimal depression of glomerular filtration rate and

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both functions was present in 8 of the dehydrated group and in only 2 of the hyperhydrated dogs There was no significant difference in blood pressure or urine volume between the 2 groups Grossly the kidneys of the dehydrated group showed edema and variable diffuse hemorrhagic changes These changes were minimal in the well hydrated group Microscopically in addition to edema and hemorrhage there were many tubules filled with eosin staining material in the dehydrated group In the hyperhydrated group these changes were minimal or absent

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General or spinal anesthesia is commonly employed clinically during aortography usually necessitates withholding oral intake for 10 to 18 hrs Further, because of the brevity of the procedure intravenous fluids are frequently given sparingly or not at all Thus a hydropenic state is reached at the time of maximal concentration of the radio opaque medium in the kidney This state of relative dehydration with associated diminished water excretion should offer both an increased concentration and exposure time of the substance in the nephron Diuresis should reduce these factors

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## METHOD

Eleven male and 3 female patients ranging in age from 11 to 61 years were studied. All had fusiform aneurysms involving the upper segment of the descending thoracic aorta and were treated by resection with homograft replacement. Observations were made of mean blood pressure, glomerular filtration rate, renal blood flow, and water and electrolyte excretion. Mean blood pressure was derived from auscultory systolic and diastolic pressures by adding one third of the pulse pressure to the diastolic pressure. Glomerular filtration rate and renal blood flow were determined by the use of inulin and para aminohippurate. Sodium and potassium determinations were made using a Beckman flame photometer. Methods and techniques have been described previously.[4-[illegible]] Three 10 min. collection periods were obtained before the patients were anesthetized and two 10 min. collection periods immediately after anesthetization. Hypothermia was then induced using an electrically controlled water-cooled blanket. Maximum reduction in temperature ranged from 29° to 33° C. Observations consisting of two 10 min. collection periods were made at variable periods somewhat above maximum hypothermia and at the point of maximum reduction in temperature. These were repeated after blood pressure had become stabilized following release of aortic occlusion and again after rewarming. Finally, three 10 min. collection periods were obtained 7 to 14 days after operation. All observations were then subjected to statistical analysis and compared with control values.† Two patients were excluded from the tables as explained below.

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†Statistical analyses by R. A. Seibert, Ph.D.

*Table 1 Effects of Anesthesia Hypothermia, and Renal Ischemia on Renal Hemodynamics and Water Electrolyte Excretion*

| PATIENT | MEAN BLOOD PRESSURE mm Hg C | AN | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | GLOMERULAR FILTRATION RATE cc./min C | AN | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | RENAL BLOOD FLOW cc./min C | AN | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | DURATION OF AORTIC OCCLUSION TIME IN MIN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H B | 99 | 93 | 94 | 102 | 95 | 89 | 98 | 86 | 78 | 81 | 64 | 46 | 47 | 99 | 868 | 748 | 568 | 328 | 709 | 560 | 1031 | 64 |
| J A | 99 | 72 | 75 | 77 | 76 | 92 | 87 | 100 | 99 | 71 | 65 | 49 | 96 | 115 | 1459 | 1378 | 1418 | 991 | 629 | 1042 | 2259 | 37 |
| M P | 70 | 70 | 70 | 77 | — | 89 | — | 88 | 59 | 50 | 50 | — | 34 | — | 772 | 390 | 435 | 442 | — | 304 | — | 47 |
| P G | 73 | 70 | 77 | 77 | — | — | 75 | 101 | 81 | 70 | 71 | — | — | 118 | 1065 | 1131 | 673 | 631 | — | — | 1163 | 63 |
| P S | 77 | 77 | 52 | 73 | 85 | 92 | 93 | 82 | 102 | 84 | 99 | 51 | 64 | 112 | 1006 | 1385 | 1261 | 1067 | 785 | 625 | 973 | 41 |
| J D | 103 | 97 | — | 87 | 99 | 100 | 101 | 101 | 89 | — | 72 | 38 | 104 | 100 | 1411 | 1215 | — | 651 | 669 | 757 | 810 | 35 |
| C S | 132 | 127 | 125 | 120 | 125 | 130 | — | 97 | 81 | 49 | 46 | 58 | 74 | — | 802 | 846 | 354 | 392 | 568 | 697 | — | 40 |
| W K. | 120 | 117 | 105 | 86 | 93 | 125 | 125 | 109 | 157 | 85 | 55 | 25 | 82 | 85 | 993 | 1256 | 948 | 631 | 545 | 705 | 866 | 53 |
| S R | 115 | 96 | 87 | 74 | 81 | 100 | 104 | 111 | 37 | 78 | 71 | 54 | 121 | 115 | 825 | 338 | 558 | 666 | 816 | 1064 | 1078 | 33 |
| D T | 82 | 97 | 86 | 112 | 100 | 81 | 113 | 32 | 38 | 63 | 41 | 31 | 27 | 50 | 485 | 579 | 592 | 463 | 522 | 383 | 424 | 47 |
| Mean | 97 | 92 | 86 | 89 | 94 | 100 | 100 | 91 | 82 | 70 | 63 | 44 | 72 | 99 | 969 | 926 | 756 | 626 | 655 | 682 | 1075 | |
| Mean % of Control | | 95 | 90 | 94 | 93 | 102 | 105 | | 93 | 88 | 75 | 54 | 79 | 115 | | 95 | 82 | 66 | 73 | 73 | 105 | |
| P Value | | 0.50 | 0.30 | 0.40 | 0.50 | 0.50 | 0.50 | | 0.50 | 0.05 | 0.01 | 0.001 | 0.20 | 0.50 | | 0.50 | 0.20 | 0.05 | 0.05 | 0.05 | 0.50 | |

| | URINE VOLUME cc/min | | | | | | | URINE SODIUM µEq/min | | | | | | | URINE POTASSIUM µEq/min | | | | | | | TEMPERATURE C° | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | AN | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | C | AN | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | C | AN | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | C | AN | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ |
| HB | 04 | 0[illegible] | 10 | 29 | 31 | 13 | 08 | 83 | 8[illegible] | 188 | 115 | [illegible] | 77 | 139 | 42 | 40 | 31 | 19 | 29 | [illegible] | 61 | NT | NT | 31 4 | 30 0 | 29 9 | [illegible] | NT |
| JA | 04 | 04 | 25 | 22 | 20 | 17 | 30 | 13 | 131 | 172 | 68 | 266 | 66 | 127 | 48 | 0 | 16 | 22 | 68 | 9[illegible] | 57 | NT | NT | 32 2 | 30 0 | 31 0 | 3 4 | NT |
| MI | 0[illegible] | 04 | 01 | 07 | — | 02 | — | 93 | 51 | 51 | 92 | — | 43 | — | 88 | 31 | 2[illegible] | 47 | — | 0 | — | NT | NT | 3 0 | 32 2 | — | — | NT |
| LC | 01 | 04 | 23 | 23 | — | — | 23 | 71 | 47 | 30 | 37 | — | — | 138 | 27 | 37 | 21 | 44 | — | — | 82 | NT | NT | 31 4 | 32 2 | — | — | NT |
| LS | 0[illegible] | 0 | 06 | 16 | 51 | 19 | 51 | 91 | 101 | 19 | 206 | 142 | 10[illegible] | 16[illegible] | 34 | 72 | 3[illegible] | 18 | 6[illegible] | 46 | 96 | NT | NT | 32 8 | 29 4 | 29 1 | 3 3 | NT |
| JD | 10 | 09 | — | 09 | 22 | 08 | 09 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | NT | NT | 33 9 | 33 2 | [illegible] | 37 8 | NT |
| CS | 20 | 14 | 13 | 07 | 31 | 13 | — | 310 | 26[illegible] | 220 | 232 | [illegible] | [illegible] | — | 102 | 91 | 16 | 37 | 68 | 91 | — | NT | NT | 31 0 | 29 0 | 3 0 | 37 8 | NT |
| RK | 04 | 27 | 4[illegible] | 09 | 11 | 27 | 19 | 88 | 140 | 91 | 70 | 162 | 18[illegible] | 100 | 50 | 9[illegible] | 10 | 37 | 42 | [illegible] | [illegible] | NT | NT | 33 3 | 31 1 | 31 8 | [illegible] | NT |
| SR | 48 | 48 | 80 | 17 | 67 | 63 | — | 101 | 38 | 83 | 6[illegible] | 196 | 12[illegible] | — | 78 | 6 | 36 | [illegible] | 71 | 8 | — | NT | NT | 32 8 | 30 0 | 31 0 | [illegible] | NT |
| DT | 06 | 06 | 16 | 33 | 10 | 14 | 20 | 121 | 7[illegible] | 82 | 87 | 12[illegible] | 63 | 116 | 22 | 22 | [illegible] | 21 | 16 | 38 | 2 | NT | NT | 31 1 | 30 3 | 01 | [illegible] | NT |
| Mean | 11 | 13 | 24 | 20 | 31 | 20 | 20 | 121 | 13[illegible] | 107 | 13[illegible] | 273 | 139 | 151 | 55 | 5[illegible] | 30 | 36 | [illegible] | 63 | [illegible] | | | | | | | |
| Mean % of Control | | 1 4 | 3[illegible] | 331 | 408 | 262 | 192 | | 12[illegible] | 92 | 128 | 210 | 128 | 151 | | 117 | 66 | 7[illegible] | 10[illegible] | 131 | 182 | | | | | | | |
| t Value | | 0 [illegible]0 | 0 20 | 0 20 | 0 0[illegible] | 0 30 | 0 20 | | 0 0 | 0 [illegible]0 | 0 50 | 0 0[illegible] | 0 0 | 0 [illegible]0 | | 0 [illegible]0 | 0 0[illegible] | 0 10 | 0 0 | 0 0 | 0 0[illegible]0 | | | | | | | |

Key to abbreviations

C=*Control observations* (average of three 10 *min* periods)

AN=Observations after the induction of anesthesia (average of two 10 min periods)

$D_1$=*Observations after initial reduction in body temperature* (*average of two 10 min period*)

$D_2$=Observations after maximum reduction in body temperature (average of two 10 min period)

$D_3$=*Observations after release of aortic occlusion* (average of two 10 min periods)

$D_4$=Observations after rewarming (average of two 10 min periods)

$D_5$=Observations 7 14 days after operation (average of three 10 min periods)

NT=Normothermic

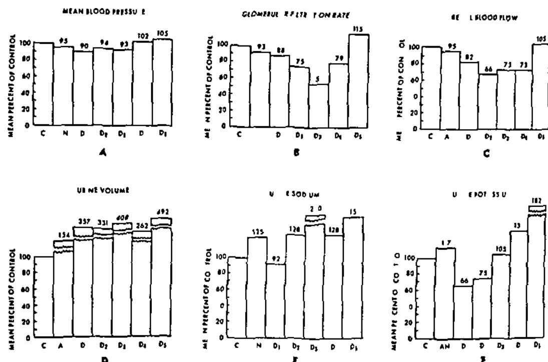



Fig 1 Diagrammatic representation of the effects of anesthesia hypothermia and renal ischemia on renal hemodynamics and water and electrolyte excretion

renal blood flow associated with a slight increase in the urinary excretion of water sodium and potassium These changes are also not significant

As body temperature was reduced glomerular filtration rate fell progressively to 75 per cent of control values (Fig 1B) Following the period of aortic occlusion which varied from 33 to 64 min there was further depression of glomerular filtration rate to 54 per cent of control values However, by the time the normothermic state was reached, glomerular filtration rate had returned toward normal and within 7 to 14 days it had reached control values

The renal blood flow paralleled the fall in glomerular filtration rate, reaching 66 per cent of control values during maximum hypothermia (Fig 1C) Following release of aortic occlusion there was immediate return to pre occlusion levels with no further change on rewarming At the end of 1 to 2 weeks normal values were obtained

Rather than the usual depression of urine volume which is associated with a fall in glomerular filtration rate under normothermic conditions urine volume increased with reduction of body temperature (Fig 1D) Following the period of aortic occlusion urine volume was further elevated The changes in sodium excretion (Fig 1E) closely paralleled those of urine volume

In contrast to the rise in water and sodium excretion associated with hypothermia there was depression of potassium excretion and this was greatly increased after return to normothermic conditions (Fig 1F)

With anesthesia hypothermia and aortic occlusion there were no significant changes in the hematocrit Plasma potassium levels were depressed slightly during the period of maximum hypothermia as were plasma sodium levels 1 to 2 weeks after operation During the period of aortic occlusion there was no measurable renal function

## DISCUSSION

Contrary to the generally held belief that anesthesia is associated with depression of renal function[6] minimal changes were noted in the patients studied This was perhaps related to the fact that light anesthesia associated with muscular relaxing agents was used However, because of the diversity of anesthetic agents employed and the small number of patients the significance of this is not known

The changes in renal hemodynamics water and electrolyte excretion during hypothermia and following temporary occlusion of the thoracic aorta appear similar to those seen in the laboratory animal[7,8] The reduction in glomerular filtration rate and renal blood flow associated with a reduction in body temperature appeared related to the hypothermic state The lack of fall in urine volume and sodium excretion associated with this depression of glomerular filtration rate was probably related to a depression of tubular function during hypothermia Additional evidence of depression in tubular function during hypothermia is demonstrated by a fall in urinary potassium excretion an active process of the tubules

Following the period of aortic occlusion, the further fall in glomerular filtration rate as well as the rise in urine volume and sodium excretion was probably related to transient glomerular and tubular depression associated with renal ischemia This ischemia did not appear to influence renal blood flow, and its overall effect appeared to be temporary as return toward normal had occurred as the normothermic state was reached The depression of glomerular filtration rate and renal blood flow remaining after rewarming was not dissimilar to that seen in the laboratory immediately following hypothermia in the dog[8]

Two of the patients studied were not included in this presentation, as renal artery occlusion unilateral in one and bilateral in the other was necessary as well as aortic occlusion Although some protection from ischemic renal damage by hypothermia can be demonstrated in the laboratory[8,9] both of these patients showed severe damage following the procedure

## SUMMARY

The effects of anesthesia hypothermia and renal ischemia on renal hemodynamics water and electrolyte excretion in 14 patients having excisional therapy of thoracic aneurysms have been presented No significant changes were seen following the induction of anesthesia Reduction in body temperature was associated with a progressive fall in glomerular filtration rate and renal blood flow This was not accompanied by significant alteration in urine volume or sodium excretion but by a depression of potassium excretion Temporary renal ischemia from occlusion of the thoracic aorta produced a further depression of glomerular filtration rate with a significant rise in urine volume and sodium excretion These effects were transient however, and return toward normal occurred with rewarming Renal function had returned to normal when final observations were made 7 to 14 days after operation

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# EVALUATION OF ARTERIAL HOMOGRAFTS FOLLOWING *SIX YEARS OF IMPLANTATION IN DOGS**

EDWARD B C KEEFER AND FRANK GLENN

Knowledge concerning the ultimate fate of an homologous arterial implant in the human should influence considerably the trend in vessel surgery The prosthesis best suited for arterial replacement even after many years of research is still a matter of contention and open to further investigation The outstanding reports of human vascular surgery in the last 8 years credit the dog as being much more than man s best friend We are all aware that it is difficult to predict human end results from animal experiments Statistically the dog lives from 8 to 12 yr and man from 60 to 80 yr Therefore the authors wish to assume that a 6 yr implantation period in the dog is the equivalent of 30 to 50 years in the human In 1952 the authors presented a report to the Forum on a 2 yr observation of a series of dogs with thoracic aorta homografts[3] Additional and more significant data following a 6 yr study is presented here

### PROCEDURE

In 1950 a litter of 6 puppies (age 3 mos wt 10 to 17 lbs) had an adult dog arterial graft of approximately the same caliber as the host s artery implanted into the thoracic aorta The homograft was inserted by an end to end anastomosis using a continuous everting mattress suture of 6 0 chromic cat gut on a minimal trauma needle The grafts had been stored for varying periods of time in nutrient media and refrigerated at 0 to 4°C according to

*Department of Surgery of the New York Hospital—Cornell Medical Center Aided by a grant from the New York Heart Assoc



Fig 1 Dog #5 Photomicrograph magnification X 38 Ossification of graft at proximal suture line Graft to right side of picture

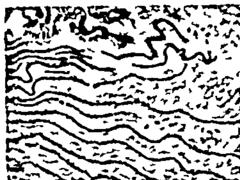


Fig 2 Dog #5 Photomicrograph magnification X 180 Calcified elastic fibers of homograft

the method then used by the New York Blood Vessel Bank and described by Gross *et al*[1] and Keefer *et al*[2]

Two years later an exploratory thoracotomy was performed on all 6 dogs Postoperative adhesions made it difficult to measure the outside diameter of the vessel These measurements have been presented in Table 2 of the previously reported 2 yr study[3] At this time angiography was employed to visualize the grafts caliber

Six years after the initial operation the dogs weighed from 41 to 60 lbs and once more were explored by thoracotomy The pleural adhesions resulting from the previous surgery made it impossible to obtain accurate measurements of the implant however the grafts were seen and palpated The lumen of these homografts were opacified by retrograde carotid arteriography

One dog died of unrelated causes 4 yr postoperatively The other 5 are alive and well

RESULTS

Owing to the technical difficulties resulting from healing scarring and adhesions it was not possible to make accurate measurements of the outside caliber of the homograft suture line and adjacent host s vessel wall Nevertheless our observations suggested that no real growth of the homograft or suture line occurred It is conceivable that some dilatation may have been present in these areas relatively soon after implantation However following

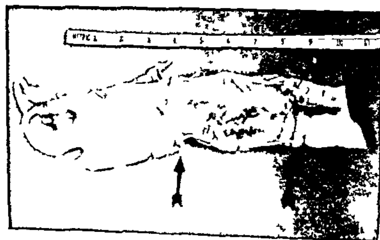


Fig 3 Dog #5 Gross autopsy specimen 4 yr after implantation Intimal surface smooth (cracks due to brittleness and opening of vessel) Arrows point to suture lines



Fig. 4. Dog #5. Photomicrograph magnification X 20. Chromic catgut suture material.

prolonged implantation, the calcification and ossification of the homograft precluded any increase in size.

No rupture, aneurysm or stricture formation has been observed in this series. These homografts have continued to function as satisfactory prostheses withstanding the normal rigors of a dog's life, such as repeated pregnancies, fights, etc.

Histological section of the thoracic aorta of dog #5 with a 1 yr. arterial implant showed several pathologic changes. No atheroma or atheromatous plaques in the intima were produced. The intima was relatively acellular and thickened over the graft and suture lines. An occasional small fleck of calcification could be seen in the intima of the graft. The media and adventitia of the graft and the immediate adjacent host's vessel wall revealed exten-

Fig. 5. Dog #7. Left side—Chest x-ray. Half moon ossification of graft between arrows not an aneurysm. Right side—Arteriography.



Fig 6 Dog #7 Operative photograph 19 6 White ossified graft not an aneurysm but due to larger donor graft

sive calcification with several large areas of ossification (Fig 1) The elastic fibers were impregnated with calcium (Fig 2) This medial calcification and bone formation of the homograft was observed to extend for a short distance into the host s vessel wall (Fig 3) The so-called absorbable chromic catgut suture was found at the suture line 6 yr postoperatively (Fig 4) The vessel lumen was not encroached upon except for minimal small mural thrombi in some areas (Fig 3)

After 2 yr of implantation dog #7 was thought to have formed a fusiform aneurysm of the graft It is now apparent that this dilatation is a bony thickening in the graft wall (Fig 5 & 6) The original anastomosis is responsible for the slight narrowing at the distal suture line and the palpable thrill over the host s distal aorta noted during the 1956 thoracotomy All the grafts

Fig 7 Dog #8 Left side—Chest x ray Right side—Arteriography Graft calcified between arrows

were palpated as hard tubes and no appreciable narrowing or dilation was observed

Chest roentgenograms showed opaque calcified homografts in all dogs (Fig 7) Arteriography envisaged a patent lumen with no stricture or obstruction to the blood flow (Figs 5 & 7)

This study will be continued as long as these animals survive

## CONCLUSION

After 6 years of implantation the degree of calcification and bone formation observed in the homograft has not impaired the function as a blood conduit The degenerative processes which developed in the arterial grafts studied in no way resembled human atherosclerosis as no atheroma were found and calcification of the elastic fibers occurred uniformly throughout *the media and adventitia with some areas of actual bone formation In these* dogs irrespective of the questionable absorbable suture material no demonstrable growth of the suture line was observed The homograft has not grown with the growth of the animal The degenerative process described above suggests that extreme care to prevent trauma and mural thrombi production on the intimal surface is an important consideration in arterial segment replacement

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# THE PROGRESSION OF EXPERIMENTAL ATHEROSCLEROSIS AFTER LUMBAR SYMPATHECTOMY*


THOMAS O MURPHY JOHN J HAGLIN AND DAVITT A FELDER


The initial salutary effect of lumbar sympathectomy in clinical atherosclerosis is well documented[1, 2] and has made this a popular procedure The later or secondary effects of this procedure in atherosclerotic individuals has not yet been clearly determined In spite of the intensive studies upon atherogenesis over the past decade there have been no reports on the effect of an autonomic denervation upon this process This is a report of an empirical approach to this problem in an experimental animal the rabbit


*From The Department of Surgery University of Minnesota Medical School Minneapolis Minnesota and St Josephs Hospital St Paul Minn Aided by funds from the Louis W and Maud Hill Family Foundation

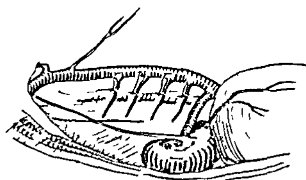


Fig 1 Lumbar sympathetic ganglia in the rabbit

METHOD

Animals were made atherosclerotic using the technique of Wacker and Hueck [3] A rabbit pellet chow was prepared by the following formula *

| | |
|---|---|
| Dehydrated alfalfa meal | 985 lbs |
| Stabilized animal fat | 15 lbs |
| Cholesterol | 8 2 lbs |
| Toasted soya flour | 11 8 lbs |

The animal fat was stabilized by adding Tennox II † Rabbits from 5 to 7 lbs were used which consumed from 50 to 150 gm of feed daily

A bilateral lumbar sympathectomy was carried out in the rabbit using intravenous nembutal anesthesia A midline abdominal incision allowed the sympathetic ganglia and trunks to be identified as thin white fibers in the retro aortic area (Fig 1) The trunks and ganglia ($L_1$ to $L_4$) were removed from the level of the renal arteries to the pelvis and a peri aortic stripping of all adventitia performed to assure an adequate sympathectomy In other sham operations the abdomen of the animal was explored and the aorta manipulated with forceps in a much more traumatic fashion than was done during the course of a sympathectomy but with care taken not to damage or sever the sympathetic trunks

The animals were divided into 6 groups as follows

1 Twelve animals were placed in cages in the laboratory and allowed to eat a normal rabbit pellet chow of similar constitution as noted but without the addition of animal fat or cholesterol These animals were not operated upon during the course of the experiment but were followed to evaluate the normal aging process of the rabbit aorta

2 Twelve animals were operated upon having a sham operation and aorta manipulation These were then placed on the normal diet as in group 1

3 Twelve animals were operated upon having a bilateral lumbar sympathectomy and peri aortic stripping below the renal arteries These were fed a normal diet

4 Twelve animals were started upon an atherogenic diet without surgery of any sort

5 Twenty four animals had a sham operation with an aortic manipulation and fed an atherogenic diet

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*Prepared by Archer Daniels Midland Company Minneapolis Minnesota

†Produced by Tennessee Eastman Corporation

6 Twenty four animals had bilateral lumbar sympathectomies and peri aortic stripping below the renal arteries Upon recovery they were fed the *atherogenic diet*

The animals were followed on these diets from 6 mo to 1 yr to evaluate the late effect of the operative procedures The only additive to the diets other than the above was the occasional feeding of fresh greens to the animals

RESULTS

The animals were followed for the development of cholesteremia and the serum cholesterol measured after the method of Bloor[4] Within 3 wk the serum of the animals on the atherogenic diet showed a marked lipemia Serum cholesterol levels in the animals eating a normal diet ran between 18.3 mg per cent to 100.2 mg per cent The animals which were fed the atherogenic diet ranged between 583 mg per cent to 1020 mg per cent blood cholesterol

*Serum Cholesterol Determinations*

| GROUP | MINIMUM | MAXIMUM | MEAN |
|---|---|---|---|
| 1 | 48.3 mg % | 100.2 mg % | 72.9 mg % |
| 2 | 50.3 mg % | 84.2 mg % | 76.3 mg % |
| 3 | 58.3 mg % | 90.3 mg % | 72.4 mg % |
| 4 | 583 mg % | 2373 mg % | 1242 mg % |
| 5 | 982 mg % | 1540 mg % | 1312 mg % |
| 6 | 6[illegible] mg % | 4020 mg % | 1320 mg % |

Animals were kept on their respective diets for 6 mo to 1 yr prior to sacrifice to assure that the lesions which developed would be beyond the acute stage The animals were sacrificed and the entire heart aorta and femoral arteries were removed and the vessels opened Tissue samples were taken for microscopic examination from the ear fore leg and hind leg

Achieving an accurate objective evaluation of the degree of degenerative changes in the arterial bed is difficult The rabbit aorta is small and the degree of involvement is a relative thing In this series we have preferred to grade the progression of the disease from zero to four as follows

Grade zero No atherosclerosis

Grade 1 Very little thickening of the intima without formation of true atherosclerotic plaques (microscopic evidence of the disease)

Grade 2 Occasional atherosclerotic plaques

Grade 3 Many atherosclerotic plaques with coalescence in many areas

Grade 4 Almost solid coalescence of the plaques in the aorta or evidence of occlusion of a major artery due to degenerative changes

**Groups 1, 2, 3** Animals in groups 1 2 3 which were fed a normal rabbit chow *showed no signs of atherosclerosis even when kept in the laboratory* for a 2 yr period All animals showed grade zero atherosclerosis

**Group 4** animals (normal animals on the atherogenic diet) showed various degrees of atherosclerosis of the aorta above the renal arteries but with little or no evidence of the disease below this level Microscopic section of the central artery of the ear showed the typical atherosclerotic lesion however the

*The Mean Degree of Atherosclerosis of the Aorta and Peripheral Vessels*

| GROUP | THORACIC AORTA | LUMBAR AORTA | PERIPHERAL VESSELS |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 |
| 4 | 2-3 | 1-2 | 0 |
| 5 | 2-3 | 2 | 0 |
| 6 | 2-3 | 3 | 2-3 |

smaller arteries and arterioles of the extremities showed only rare lesions The mean involvement of the group was graded a 2 to 3 above the renal arteries and 1 to 2 below this level

**Group 5** animals ( sham operation with an atherogenic diet) showed similar atherosclerotic degeneration of the aorta and main arterial branches above the renal arteries Below the renal arteries there was a slight increase in the degree of atherosclerosis in the area of the trauma to the aorta There was no change in the peripheral vessels The mean involvement of the group was grade 2 to 3 above the renal arteries and 2 below the renal arteries

**Group 6** animals (sympathectomy and atherogenic diet) showed severe atherogenic degeneration above and below the renal arteries with extensive atherogenic degeneration involving the superficial femoral arteries as far as *they could be examined (Fig 4) In 2 of the 21 animals* the disease of the terminal aorta was enough to cause thrombosis of this area

Microscopic tissue section showed numerous areas of small vessel involvement in the thigh sections but only rare involvement of the biopsy from a comparable area in the foreleg




Fig 2 The lumbar aorta showing the atherosclerotic plaques stained with scarlet red (*Sudan IV*) *stain* (A) Normal aorta (B) Atherogenic diet without surgery (C D) Atherogenic diet with lumbar sympathectomy

### DISCUSSION

The experiments of Taylor Baldwin and Hass[5] and of Baldwin *et al*[6] have shown that areas of arterial injury are predisposed to the formation of atherosclerosis in hypercholesterolemic rabbits The normal reparative process of a damaged artery was the formation of a new arterial wall within a proliferating intima When such damage was associated with hypercholesterolemia there was a localized deposition of lipids and cholesterol with formation of foam cells

In view of these data to evaluate only the effect of the sympathectomy it was necessary to carry out the sham operation with manipulation and traumatization of the aorta in a manner similar to that done during the sympathectomy and peri aortic stripping The finding of atherosclerotic changes in the peripheral arteries of the extremities was rare in the control animals However in the sympathectomized animals such changes were noted with frequency We may conclude that the more advanced degenerative changes of atherosclerosis in the aorta and peripheral vessels is somehow related to the sympathectomy

The cause and effect relationship in this experiment is not clear There is evidence that the changes of physical stresses upon the arterial wall was related to the progression of atherosclerosis It has been demonstrated that following sympathetic ganglionectomy there is a lessening of the peripheral resistance in the area affected and thus a higher rate of blood flow into this area Furthermore, we may speculate that there may be a weakening of the muscular wall of the artery due to paralysis of the smooth muscle fibers It is our contention that these 2 factors contribute to the increase in degeneration found in the sympathectomized animals

### SUMMARY AND CONCLUSIONS

A comparison of the degree of atherogenic degeneration of the rabbit aorta and peripheral arteries was made between normal and sympathectomized animals It seems conclusive that trauma to the abdominal aorta increases the degree of atherosclerosis and further that a sympathectomy of the arterial bed causes an even greater degree of atheromatous degeneration in both the aorta and the distal arteries of the extremities

It is difficult to correlate these findings with human arteriosclerosis However it is suggested that these data may cause one to re-evaluate the indications for sympathectomy in a patient who has a disturbed lipid metabolism

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# GENERAL AND DIFFERENTIAL HYPOTHERMIA (INTRAPERITONEAL) IN PREVENTION OF ISCHEMIC SHOCK AND PARAPLEGIA FOLLOWING TEMPORARY OCCLUSION OF THE THORACIC AORTA*

WILLIAM M PARKINS MAX BEN AND HARRY M VARS

The data we wish to report at this time are representative of one aspect of a continuing study designed to (1) more clearly define the relative roles of the liver and intestinal tract in the pathogenesis of ischemic shock and to (2) determine the efficacy of generalized and differential hypothermia in combating the irreparable tissue damage induced by general and regional occlusions of the circulation

This presentation will be confined to current results from the use of hypothermia induced intraperitoneally and of chlorpromazine in preventing ischemic shock and paraplegia which follows temporary occlusion of the thoracic aorta

## METHOD

Healthy adult dogs were anesthetized with pentobarbital (25 mg /kg ) intravenously supplemented by additional nembutal as required In the chlorpromazine group 5 mg /kg was used Three mg /kg I V was followed by one half the usual amount of nembutal required for anesthesia In addition 1 mg /kg chlorpromazine was given 1 hr after the initial dose and 1 mg /kg was repeated just prior to aortic occlusion

Techniques of placement of catheters for blood sampling blood pressure and the positioning of a specially designed balloon catheter for occlusion between the 8th and 10th intervertebral space were previously described [1]

The intraperitoneal method of differential cooling previously reported[1] involved the recirculation of ice cold saline through the opened abdomen during the period of occlusion The cooling procedure has been simplified in the present experiments by the single rapid intraperitoneal injection of iced saline (65 cc /kg ) supplemented by an ice pack over the lower abdomen When the cold saline (0 to 5°C ) was administered within 2 min after the aortic occlusion a differential cooling persisted with the intestine being cooled to about 20 to 26°C while the oral temperature was reduced to a range of 30 to 32 C Without obstruction of splanchnic blood flow the cold was equilibrated throughout the body and within 1 hr a generalized hypothermia to about 25°C was obtained The hypothermic animals were rewarmed to about 35°C within 2 to 3 hr by forced air heat under and around the body supplemented by a heat bulb over the abdomen

## RESULTS

Occlusion of the thoracic aorta at T 8 to 10 reduced the arterial pressure distal to the obstruction to an average level of 16 mm Hg The tolerance of the normothermic controls to 30 60 and 120 min of occlusion (T 8 to 10) is shown in Table 1 The incidence of paraplegia due to spinal cord damage

From the Harrison Department of Surgical Research Schools of Medicine University of Pennsylvania Philadelphia Supported in part by contract DA 49 007 MD 511 between the Department of the Army and the University of Pennsylvania

*Table 1 Temporary Occlusion of the Thoracic Aorta (T8–10) in Normothermic Control Dogs*

| OCCLUSION TIME (MINUTES) | NUMBER OF DOGS | SURVIVING HRS AFTER RELEASE OF OCCLUSION 24 | 72 | NUMBER PARAPLEGIC |
|---|---|---|---|---|
| 30 | 10 | 8 | 8 | 4 |
| 60 | 10 | 4 | 4 | 4 |
| 120 | 10 | 0 | 0 | — |

*Table 2 Temporary Occlusion of the Thoracic Aorta (T8–10) in Hypothermic Dogs With and Without Chlorpromazine*

| OCCLUSION TIME (MINUTES) | METHOD OF INTRAPERITONEAL COOLING | NUMBER OF DOGS | SURVIVING HRS AFTER RELEASE OF OCCLUSION 24 | 72 | NUMBER PARAPLEGIC |
|---|---|---|---|---|---|
| 60 | Differential | 5 | 5 | 5 | 2 |
| 120 | Differential | 5 | 5 | 5 | 3 |
| 120 | Generalized | 10 | 7 | 7 | 0 |
| 120 | Generalized + chlorpromazine | 10 | 8 | 8 | 0 |

and the mortality from ischemic shock is evidently a function of the duration of the reduced blood flow and oxygen supply The degree and duration of the post occlusion hypotension and the extent of the progressive hemoconcentration which followed was increased after the more prolonged occlusion The most severe mucosal sloughing and hemorrhage into the lumen of the intestine and bloody mucous discharge from the rectum was consistently observed in the 1 and 2 hr normothermic animals which died within 2 to 6 hr after occlusion

The efficacy of differential hypothermia as compared with generalized precooling with and without chlorpromazine is shown in Table 2 By the intraperitoneal injection of 0 to 5°C saline immediately after occlusion of the thoracic aorta a greater reduction in temperature of the intestine was obtained, and all animals survived an interval of ischemia which was invariably fatal to normothermic dogs About half of the differentially cooled animals which survived 1 and 2 hr of occlusion were paralyzed In the generally precooled group the temperature of the blood and presumably the spinal cord was below 28 C and no evident paraplegia occurred In the differentially cooled dogs the hypothermia induced after occlusion was localized and afforded inadequate protection for the spinal cord in the first hour of ischemia

The protection against shock and death was somewhat less effective in the precooled dogs since 5 of the 20 precooled did not survive No significant difference was observed between the 10 precooled dogs which received chlorpromazine and the 10 dogs without chlorpromazine except as to the nembutal requirements for anesthesia Bloody diarrhea and mucosal sloughing was minimal and the post release hypotension and hemoconcentration were much less severe in the surviving dogs of both hypothermic groups

DISCUSSION

Pontius and associates[2] found hypothermia induced by surface cooling to be highly effective in preventing the paraplegia resulting from cord damage due to high level occlusion of the thoracic aorta but was of little influence upon the mortality. Essentially the same result was reported in our published experiments in which generalized precooling to 30°C was induced by external surface cooling or by internal blood cooling.[1] However, when the rectal temperature was reduced to about 25°C by blood cooling 7 of 11 dogs survived the 1 hr occlusion without paralysis. Burch *et al*[3] report survival of dogs after 60 min of occlusion of the thoracic aorta in which the liver was cooled by coils placed about the liver. Raffucci *et al*[4] were able to increase the tolerance time of dogs to temporary ligation of the afferent circulation of the liver with generalized hypothermia induced by surface cooling. We reported last year our negative results with liver arterialization and the moderate degree of protection against the ischemic shock obtained with differential hepatic hypothermia.

Though the tolerance time of the spinal cord to occlusion of the thoracic aorta has been markedly increased by various methods of generalized hypothermia, a far greater degree of protection against the ischemic shock has resulted from intraperitoneal precooling and especially the rapid differential cooling of the intestine by cold saline.

SUMMARY AND CONCLUSION

Both the method and degree of cooling affect significantly the protection afforded by hypothermia in the ischemic shock and paraplegia resulting from occlusion of the thoracic aorta. Thus generalized hypothermia to 30°C or lower induced prior to occlusion by surface, blood or intraperitoneal cooling, though highly effective in preventing paraplegia, may have little effect upon the shock syndrome and mortality.

Generalized precooling by the intraperitoneal administration of iced saline greatly increased the tolerance time to occlusion as judged by the 15 of 20 dogs which survived 2 hr of ischemia without paralysis. Differential cooling of the viscera by rapid injection of 0 to 5°C saline intraperitoneally just following occlusion was the most efficacious in reducing symptoms and mortality in this type of ischemic shock. Post-occlusion hypotension, hemoconcentration, bloody diarrhea and sloughing of the intestinal mucosa are minimal or absent and dogs survived a 2 hr interval of occlusion which was invariably fatal to normothermic controls.

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# VASCULAR CHANGES AND DIRECT TISSUE EFFECTS IN SEVERE COLD INJURY*

W L OGILVY M A ENTIN AND D R WEBSTER

*This investigation was undertaken to observe and assess the effect of cold* directly on tissue and the vascular changes that occur on exposure to cold and thawing

In our experiments, the dog was the animal used and the part subjected to the cold injury was the thigh

The investigation was divided into 2 experiments but the animals were exposed to the same degree of cold and for the same duration throughout the whole investigation In the first experiment an attempt was made to evaluate the physiopathological changes in cold injury by means of a skin grafting procedure and in the second experiment an assessment of blood flow in the cold injured area was made by means of radio active phosphorus

## METHOD

**Experiment 1** Twenty dogs were used in this part of the investigation Intravenous nembutal was the anesthetic used throughout

When anesthetized the dog had one hind leg and the corresponding side of the body clipped and shaved A split thickness strip of skin was then removed from the dog s side and this strip of skin was divided into four sections A, A1, B and C The leg with the foot protected by an impervious dressing was then immersed in a tank containing 95 per cent ethyl alcohol and $CO_2$ ice at —20°C for 1 hr This allowed only an area of the thigh to be exposed to the freezing mixture

The 4 sections of skin were disposed as follows A and A1 were kept at room temperature and B and C were placed in separate rubber gloves the cuffs tied securely and immersed in the alcohol ice mixture along with the leg

After 1 hr immersion a split thickness section of skin section D was removed from the lateral aspect of the thigh Skin section B was thawed by immersion in water at 12 C for 5 min , and section C was allowed to thaw at room temperature

The 5 sections of skin A A1 B C and D were then re applied as auto grafts and the 5 areas on the dog s side and leg then represented the following Area A—Skin frozen *in situ* (section D) then excised and re applied to a normal bed Area A1—Normal skin (section A1) on a normal bed This was a control normal skin graft Area B—Excised skin (section B) frozen then rapidly rewarmed and re applied to a normal bed Area C—Excised skin (section C) frozen then slowly rewarmed and re applied to a normal bed Area D—Normal skin (section A) on a damaged bed (Fig 1)

## RESULTS

The survival of the skin grafts was assessed clinically after 14 days During this time however 6 of the dogs had to be excluded from the series because

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*From the Department of Experimental Surgery McGill University Montreal Canada

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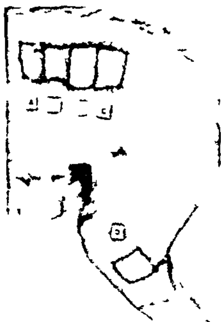


Fig 1 The side and thigh of dog 49 showing the distribution of the five sections of the skin after they have been sutured into the designated areas (see text)

of technical difficulties or death This left 14 dogs for assessment and the skin graft survivals were as follows Area A—86 per cent Area A1—90 per cent Area B—0 per cent Area C—28 per cent Area D—7 per cent

The grafted areas were also biopsied 14 days after the cold injury and although the histological sections showed the structural changes present at this time they did not contribute any information as to why some grafts survived and others did not

**Experiment 2.** The dogs were anesthetized with intravenous nembutal then both hind legs were clipped and shaved One leg with the foot protected was then immersed in the alcohol ice mixture at —20°C for 1 hr following which it was allowed to thaw at room temperature The following day 10 cc of blood were removed from the dog and incubated with 100 microcuries of radio-active phosphorus at 37 C for 30 min This blood was then re injected into the dog and $P^{32}$ activity in the injured leg and normal leg was recorded Readings were taken at 5 15 30 and 60 min following injection and daily thereafter until any sloughing of skin occurred which was usually around the fourth day At the end of 14 days the total area of tissue loss was measured on the cold injured limb

RESULTS

Sixteen dogs were used in this part of the investigation 3 of which had to be excluded from the series Recordings of $P^{32}$ activity in the control and injured legs were made in the remaining 13 dogs

The area of tissue loss after 14 days was very variable the variation being from 9 76 to 43 97 square inches

It was found that the greater the initial difference in $P^{32}$ activity between the control and cold injured limbs the greater was the subsequent tissue loss after a 14 day period (Fig 2 A and B)

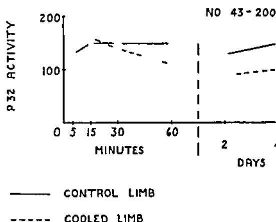



Fig 2 A The graph of $P^{32}$ activity in the dog in which the final loss of tissue in the cooled limb was 11 07 square inches at the end of 14 days

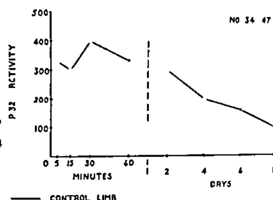



Fig 2 B The graph of $P^{32}$ activity in the dog which showed an extensive loss of skin at the end of 14 days (43 97 square inches)

DISCUSSION

The technique used in the skin grafting procedure was apparently adequate since the normal skin graft control (area A1) showed a 90 per cent survival rate

The skin graft on areas A and D may be considered together The skin applied to area A had previously been frozen *in situ* and survived in 86 per cent of cases even when the skin remaining on the cold injured limb became necrotic Although showing some histologic structural changes which were presumably due to the direct action of cold it is felt that these survived because they were saved from the vascular sequelae of edema stasis ischemia and anoxia which occur during and after thawing

The skin applied to area D was normal skin and it survived only partially in one instance on an animal which showed a minimal amount of tissue loss from the freezing injury The low survival rate appears to be due to the fact that the vascular changes following thawing are such that even normal skin cannot be kept alive when applied to this damaged bed

The survival of grafts on areas B and C are seen to be much lower than on area A although a similar bed was used in each case The skin applied to areas B and C however was frozen as free skin grafts and this may indicate that these sections received a more severe injury than the skin frozen *in situ* and applied to area A The latter may have been warmed slightly from the deeper tissues and in this way may not have dropped to as low a temperature as the isolated skin The skin on areas B and C must obviously have been damaged directly by cold and the different survival rates on B and C must correlate with the post freezing handling of these grafts

The $P^{32}$- activity in the legs of the dogs in Experiment 2 is taken as an index of the blood flow to the skin of the normal and cold injured areas These recordings were relative and applied only to individual dogs but by representing the readings in graphic form it was very evident that a variation in discrepancy of blood flow in the control and injured limbs was present It was interesting to note that in a 14 day follow up the dogs with the greatest tissue loss were those which had shown the greatest discrepancy in blood flow between the limbs in the first days immediately after sustaining the cold injury

SUMMARY AND CONCLUSIONS

Assessment of survival of frozen skin grafts thawed at room temperature and subsequently transplanted on normal beds suggests that direct injury by cold produced irreversible damage The survival rate was even lower when rapid thawing was used

Skin frozen *in situ* however survived well when transplanted to a normal bed while the remaining skin invariably became necrotic. This suggests that the direct injury by cold was not severe enough to destroy the tissue but when the secondary sequelae due to the vascular changes on thawing were added necrosis became inevitable

Using radio active phosphorus the blood supply to the cold injured part was determined It was possible to correlate the amount of initial difference in $P^{32}$ activity in the frozen and control extremities with the subsequent extent of tissue necrosis that occurred

It is concluded therefore from this investigation that severe cold can produce damage directly when applied to isolated tissue With skin *in situ*, however cold can produce structural changes but the factor of ischemia during and after thawing is an important etiologic agent in the production of massive tissue necrosis

# *Esophagus, Stomach and Small Bowel*

## UTILIZATION OF THE ILEOCECAL VALVE AS A SUBSTITUTE FOR THE CARDIO ESOPHAGEAL SPHINCTER *

### *An Experimental Study*

JOHN S NAJARIAN, DWIGHT H MURRAY, JR C D BUSTER AND ORVILLE F GRIMES

Considerable experimental and clinical investigation has been directed toward elimination of the complication of esophagitis that follows excision of the esophagogastric junction Previous studies have been directed primarily toward either the surgical construction of a valve with autogenous tissue[1, 2] or the interposition of a segment of jejunum between the esophagus and stomach[3] The latter procedure has protected the esophagus both clinically and experimentally but resulted in some degree of jejunitis[3]

In order to make a more direct approach to the problem, the following series of experiments was done in which a naturally occurring intestinal valve was substituted for the excised or pathologically destroyed cardiac sphincter mechanism This experimental study was designed to evaluate the protection afforded the esophagus by interposition of the ileocecal valve and the effect of acid peptic digestion on the interposed colon

#### METHOD

Eighteen adult mongrel dogs were used in this study Each animal was given 1 gm of neomycin sulfate daily by mouth for 2 days prior to the operation All operations were performed with general anesthesia produced by intravenous administration of pentobarbital sodium solution and strict aseptic precautions Excision of the esophagogastric junction and a supra diaphragmatic vagotomy were performed through a left combined thoraco abdominal incision The terminal esophagus was resected superiorly at the level of the left pulmonary vein The ileocolic segment including 20 to 25 cm of terminal ileum and approximately 2 to 3 cm of colon was isolated and the ileocolic vessels were preserved as its blood supply (Fig 1a) This ileocolic segment was then transposed through the greater omentum to a retrogastric position (Fig 1b) and anastomosis was accomplished between the terminal ileum and esophagus (Fig 1c) The colon was anastomosed to the stomach at the site of excision of the cardioesophageal junction (Fig 1d)

Because of the vagotomy Finney pyloroplasty was done in 8 dogs (Group 1) and Rammstedt pyloromyotomy in 8 animals (Group 2) in order to obtain adequate gastric emptying Postoperatively the animals were given 600 000 units of penicillin and one half gm of streptomycin daily for 4 days In

* From the Surgical Research Laboratories of the University of California School of Medicine San Francisco Supported by the Christine Breon Fund for Medical Research

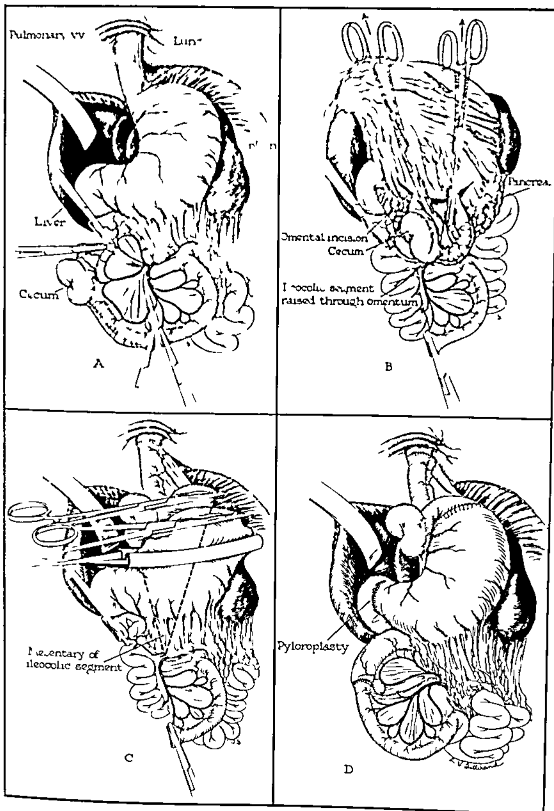



Fig 1 Illustrations of the technique of substitution of the cardioesophageal junction with the ileocecal valve (A) The dotted lines indicate the extent of excision of the terminal esophagus and cardia of the stomach The ileocolic segment has been isolated with preservation of the ileocolic vessels (B) The greater omentum has been elevated and the ileocolic segment transposed through the lesser sac (C) The esophagoileal anastomosis has been completed and the colon gastric anastomosis is about to be done (D) The colon gastric anastomosis and a Finney pyloroplasty have been completed Gastrointestinal continuity is established with an end to end ileocolostomy

travenous fluids were administered for the first 3 days after which fluids were permitted by mouth By the fifth day all the dogs were taking the regular kennel diet diluted with 1 part milk

Competency of the ileocecal valve was determined 30 days postoperatively at which time the animal was fed a barium meal through a gastric tube and then anesthetized with pentobarbital sodium With the dog in the Trendelenberg position the possibility of reflux of barium from the stomach into the esophagus was tested by roentgenologic examination

After a 6 wk postoperative recovery period all of the animals except 2 were given daily subcutaneous injections of 30 mg of histamine phosphate in oil and beeswax[4] for 45 days They were then sacrificed for examination and photography of the gastrointestinal tract Two of the animals which are being maintained for long term study were not given prolonged histamine stimulation

The competency of the ileocecal valve was again determined at autopsy by inflation of the excised stomach with water The transposed valve was considered to be competent if it withstood an intragastric pressure of 50 cm of water

Histological sections were taken from all the specimens through 5 representative areas These included (1) the esophagus 4 cm proximal to the esophago ileal anastomosis (2) the esophago ileal anastomosis (3) the center of the ileal segment (4) the ileocecal valve and (5) the cologastric anastomosis If any other areas of inflammation or ulceration were observed microscopic sections were obtained

RESULTS

After prolonged stimulation with daily doses of histamine no evidence of either gross or microscopic inflammation was found in the terminal ileum proximal to the ileocecal valve The short segments of colon distal to the valve were inflamed to varying degrees in 6 out of 16 dogs and were more resistant to acid peptic digestion than either the stomach or the duodenum (Fig 2)

Inflammation of the transposed segment of colon occurred only one half as frequently in Group 1 (Finney pyloroplasty) as in Group 2 (Rammstedt pyloromyotomy) This may be attributed to the more adequate gastric drainage accomplished with the Finney pyloroplasty There were only 2 cases of gross ulceration in the transposed segments of colon and in both cases an inadequate stoma between the colon and stomach was found at autopsy Because of the fact that the ulcers in both instances occurred on the wall of the colon directly opposite the small colon gastric stoma it appears possible that the ulcers may have resulted from the action of the jet stream of acid chyme on these particular areas of transposed colon

Roentgenologic examination *in vivo* or water pressure tests (below 50 cm of water) on the specimens at autopsy indicated that all the ileocecal valves were competent Pressure exceeding 50 cm of water resulted in incompetence of the valve This finding was confirmed by the animals ability to vomit after administration of morphine (This may be important because while the cardioesophageal sphincter mechanism must prevent reflux it must also be capable of relaxing when vomiting occurs)

The length of terminal ileum that could be mobilized without difficulty



Fig 2 Photograph of a pathology specimen showing severe gastritis and duodenitis with both gastric and duodenal ulcers after 22 days of stimulation with histamine No evidence of gross or microscopic inflammation was found proximal to the ileocecal valve

varied between 15 and 25 cm and the segments could be used for esophageal replacement as high as the mid esophagus The vascular anatomy of the ileocolic region invariably allowed its mobilization Near the ileocecal valve the ileocolic artery divided into a colic branch which passed upward on the ascending colon and an ileal branch that ran along the mesenteric border adjacent to the terminal ileum and joined the superior mesenteric artery Isolation of a long ileal segment together with the cecum and very little accompanying mesentery was thereby accomplished without difficulty

The 2 dogs being maintained for long term study are now 11 months post operative and show no evidence of esophagitis as determined by esophagoscopy

SUMMARY

The transposed ileocecal valve was utilized as a replacement for the esophagogastric junction in 18 dogs The effects of long periods of histamine stimulation on the interposed ileocolic segments were observed No evidence of inflammation was found proximal to the ileocecal valves and the small colonic segments distal to the valves were more resistant to acid peptic digestion than either the stomach or duodenum

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# THE PREVENTION OF ESOPHAGITIS AFTER ESOPHAGOGASTROSTOMY IN DOGS BY AN INTERPOSED PEDICLED PYLORUS*

PAUL T McGANNON CARL WILLIAMS, AND STANLEY R FRIESEN

Reflux esophagitis may occur as a disabling complication following operative procedures in the esophagogastric area for both benign and malignant diseases [1 - 2] Normally the esophagogastric junction is remarkably competent in protecting the susceptible esophageal mucosa from the injurious action of the gastric chyme [3] This protection has been attributed to a number of components of the esophagogastric junction, and include the diaphragmatic pinchcock,[4] esophagogastric angle and the gastro esophageal sphincter mechanism [6] *In the dog continence of the esophagogastric junction is* dependent primarily upon an intrinsic sphincter, independent of normal diaphragmatic attachments [7]

There have been several experimental and clinical operative procedures described for the prevention or treatment of esophagitis Watkins advocates the reconstruction of the esophagogastric junction, when possible to restore continence in this area [8] Ellis has used the combined procedure of antrectomy cardiectomy and vagotomy [9] Burford and Lischer have reported a series of patients with esophagitis without stricture treated simply with Finney pyloroplasty [10] Surgically devised valves have been used to prevent esophagitis [11 - 12] An isoperistaltic segment of jejunum to simulate sphincteric action has been substituted for the esophagogastric junction experimentally and clinically [13] It would be desirable to substitute a true sphincter for the esophagogastric sphincter to protect the esophagus when this junction is diseased or surgically excised It is the purpose of this presentation to report the results of the interposition of the pylorus between the esophagus and stomach as well as pylorectomy in the prevention of experimentally produced esophagitis in dogs

The pylorus is suitable as a substitute by virtue of the fact that it is a proven anatomic and physiologic sphincter in close proximity to the esophagogastric area which can be isolated preserving its blood and nerve supply McCrea has described a special branch of the vagus from the anterior vagal trunk to the pylorus [14] Wheelon and Thomas have shown that even when denervated the pylorus is capable of rhythmic tonicity [15] The

*From the Department of Surgery University of Kansas Medical Center Kansas City Kansas Supported by funds from the Surgical Developmental Fund University of Kansas School of Medicine

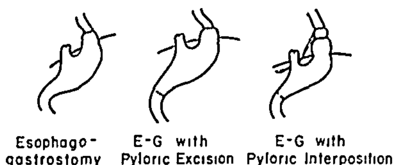



Fig 1 Diagrammatic representation of experimental preparations in Series 1 2 and 3

pylorus of the dog isolated with its neurovascular pedicle and resutured to its normal gastric and duodenal attachments appears to function normally

## METHOD

Adult mongrel dogs ranging in weight from 10 kg to 17 kg were used in 3 series of experiments (Fig 1) *Series 1* Seventeen dogs were subjected to transsection of the terminal esophagus 3 cm above the diaphragm with inversion closure of the distal end and anastomosis of the proximal end to the fundus of the stomach *Series 2* In 8 dogs the esophagogastric junction was similarly bypassed by esophagogastrostomy and in addition pyloric excision with gastroduodenostomy was carried out *Series 3* In 13 dogs the distal esophagus was similarly divided the distal end closed and the isolated pylorus with its neurovascular pedicle was interposed between the proximal esophagus and the fundus of the stomach Distal continuity was established by gastroduodenostomy

All operations were performed with sterile technique using intravenous sodium pentothal anesthesia (15 mg /lb of body weight) Respiration was controlled with endotracheal oxygen and an automatic respirator The left transthoracic incision through the ninth interspace was used in each instance Anastomoses were in 2 layers with a running 3 0 plain catgut mucosal suture and interrupted 1 0 silk seromuscular sutures The diaphragm was incised anteriorly apart from the esophageal hiatus to permit adequate exposure of the pylorus The pylorus with a small margin of antrum was isolated together with the right gastric artery or its branches in the gastrohepatic ligament and rotated 90° laterally when interposition was carried out Care was taken not to constrict the blood supply to the pedicled pylorus in the closure of the diaphragm about the gastric fundus The vagus trunks were left intact in all series of dogs

All animals in the 3 series were given only water for 2 days followed by a liquid food preparation and started on solid food at 1 wk postoperatively All dogs were weighed at regular intervals Esophagoscopy under general anesthesia was done at intervals in many of the dogs Antibiotics were given for one week At death or sacrifice of the animals gross and microscopic observations of the esophagus stomach and duodenum were recorded

## RESULTS

Series 1 (See Table 1) Sixteen of 17 dogs which had bypass esophagogastrostomy without pyloric excision or interposition had developed esophagi

*Table 1 Series 1 Dogs with By pass Esophagogastrostomy Only*

| DOG NO | DAYS | FINDINGS | REMARKS |
|---|---|---|---|
| 119 | 14 | No esophagitis | Died |
| 120 | 20 | Severe esophagitis with perforation | Died |
| 19 | 28 | Esophageal erosion | Died |
| 115 | 29 | Moderate esophagitis | Died |
| 103 | 30 | Esophagitis | Died |
| 76 | 39 | Severe ulcerative esophagitis | Died |
| 70 | 40 | Esophagitis | Died |
| 133 | 56 | Esophagitis | Died |
| 43 | 64 | Mild esophagitis | Sacrificed |
| 47 | 65 | Moderate esophagitis | Sacrificed |
| 52 | 68 | Esophagitis | Died |
| 124 | 68 | Moderate esophagitis | Sacrificed |
| 114 | 76 | Moderate esophagitis | Died |
| 118 | 78 | Moderate esophagitis | Sacrificed |
| 113 | 78 | Moderate esophagitis | Sacrificed |
| 14 | 79 | Severe esophagitis | Sacrificed |
| 117 | 82 | Moderate esophagitis | Sacrificed |

*Table 2 Series 2 Dogs with By pass Esophagogastrostomy and Pylorectomy*

| DOG | | FINDINGS | | |
|---|---|---|---|---|
| NO | DAYS | ESOPHAGOSCOPY | AUTOPSY | REMARKS |
| 16 | 25 | Esophagitis | No esophagitis | Died |
| 73 | 30 | Esophagitis | Mild esophagitis | Sacrificed |
| 122 | 40 | Mild esophagitis | No esophagitis | Died |
| 128 | 42 | Esophagitis | No esophagitis | Sacrificed |
| 14 | 60 | Esophagitis | No esophagitis | Died |
| 124 | 68 | Mild esophagitis | Moderate esophagitis | Sacrificed |
| 126 | 69 | Esophagitis | No esophagitis | Sacrificed |
| 84 | 81 | Esophagitis | No esophagitis | Sacrificed |

tis when sacrificed 11 to 82 days after operation The one dog which did not develop esophagitis died 14 days after operation because of thoracic empyema due to operative contamination

**Series 2** (See Table 2) Eight dogs with by pass esophagogastrostomy and pylorectomy had evidence of esophagitis on repeated esophagoscopic examinations at some time prior to sacrifice while only 2 of the 8 had esophagitis as a gross finding at the time of autopsy The transitory esophagitis was mild to moderate in degree and was noted by several observers The average weight loss in this series of dogs was 34 per cent

**Series 3** (See Table 3) None of the 13 animals with the pedicled pylorus interposed between the esophagus and stomach had evidence of esophagitis 14 to 91 days following the procedure No esophagitis was seen on esophagoscopy even though this procedure was done repeatedly in 4 of the dogs One animal died 29 days postoperatively from a perforated duodenal ulcer

*Table 3 Series 3 Dogs with Interposition of the Pedicled Pylorus Between the Esophagus and Stomach By pass*

| DOG | | FINDINGS | | |
|---|---|---|---|---|
| NO | DAYS | ESOPHAGOSCOPY | AUTOPSY | REMARKS |
| 129 | 14 | Not done | No esophagitis | Died |
| [illegible] | 18 | Not done | No esophagitis | Sacrificed |
| 78 | 19 | Not done | No esophagitis | Sacrificed |
| 70 | 20 | Not done | No esophagitis | Sacrificed |
| 98 | 20 | Not done | No esophagitis | Died |
| 15 | 26 | Not done | No esophagitis | Sacrificed |
| 71 | 29 | Not done | No esophagitis | Died perforated duodenal ulcer |
| 57 | 34 | No esophagitis | No esophagitis | Sacrificed |
| [illegible] | 46 | No esophagitis | No esophagitis | Sacrificed |
| [illegible] | 83 | No esophagitis | No esophagitis | Sacrificed |
| 99 | 84 | No esophagitis | No esophagitis | Sacrificed |
| 132 | 87 | No esophagitis | No esophagitis | Sacrificed |
| 130 | 91 | No esophagitis | No esophagitis | Sacrificed |

no esophagitis was found The average weight loss in this series was 18 per cent

A significant number of dogs died in the immediate and early postoperative period (not listed in Table) because of gangrene of the pylorus due to technical errors in maintaining a good blood supply through the vascular pedicle Constriction of the pedicle at the level of the diaphragm can be avoided by meticulous attention to closure of the diaphragm about this pedicle

## DISCUSSION

When the pylorus was placed between the esophagus and the stomach by passing the esophagogastric sphincter there was no evidence whatsoever of esophagitis The results of this study suggest that this protection may be due in part to absence of pyloric resistance at the distal end of the stomach and in part to its interposition separating the stomach from the esophagus Although pylorectomy without interposition demonstrates a significant decrease in the severity and incidence of esophagitis the addition of pyloric interposition yields a uniform protection The weight loss in the pylorectomy group of dogs was noticeably greater than in the series when pyloric interposition was added The reason for this difference is not known The mortality rate on the other hand is greater in the group in which the pylorus is pedicled and interposed The significant though not complete protection attending pylorectomy in this study supports the clinical experience of Lischer and Burford that Finney pyloroplasty benefits patients with esophagitis

Just prior to the time of sacrifice of the dogs with the interposed pedicled pylorus a number of these animals were anesthetized so that observations of the pylorus in this location could be made Definite contractions of the pylorus could be seen which also responded to direct electrical stimulation but not to vagal trunk stimulation Detailed studies of pyloric function are being done Minimal organic obstruction at the interposed pylorus was noted in only one dog

It is interesting that 1 dog (No 71) with pyloric interposition died 29 days following operation because of a perforated duodenal ulcer This dog had appeared to be in good health and had lost only 2 per cent of its body weight No other acid peptic changes were noted in the stomach or duodenum of any of the other dogs It would be interesting to know if chronic histamine in beeswax stimulation would increase the incidence of esophagitis in the experimentally protected animals

## CONCLUSIONS

1 Esophagitis is a common finding in dogs following esophagogastrostomy which bypasses the esophageal gastric junction

2 When a pylorectomy is combined with the esophagogastrostomy esophagitis of a transitory nature is seen to develop which subsides in time

3 No evidence of esophagitis was noted in the dogs with a pylorus interposed between the esophagus and gastric bypass

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Addendum At the time of the presentation of this paper 13 dogs were included in Series 2 of the experiments 4 dogs *of which demonstrated* esophagitis at the time of autopsy

# THE SIGNIFICANCE OF INNERVATION IN THE FUNCTION OF THE GASTRIC ANTRUM*

H. A. Oberhelman, Jr., Stanley P. Rigler and Lester R. Dragstedt

It has been established both experimentally and clinically that the gastrin mechanism remains active after exclusion of the gastric antrum from the remainder of the stomach providing that distension of the antrum by regurgitated food and intestinal content occurs.[1] However, total isolation of the denervated antrum abolishes the gastric phase of secretion.[2] It seemed of interest to study the behavior of the antrum with intact innervation after total exclusion from the gastro-intestinal tract.

## METHOD

Heidenhain pouches were constructed in 4 dogs. After recovery from the operative procedure control studies were obtained. The antrum was then excluded from the body of the stomach by a mucosal bridge leaving the entire seromuscular layer, lesser curvature structures, and nerve supply intact. The pylorus was divided and a cutaneous fistula constructed from the proximal end. Gastrointestinal continuity was re-established by gastroduodenostomy (Fig. 1).

Following recovery from the operation quantitative daily collections of gastric juice were resumed using the methods previously described from this laboratory. The mean daily output of hydrochloric acid during a 30 day collection period was expressed in mEq. The secretory response to feeding a standard meal, fasting for a 3 day period, and the intravenous administra-

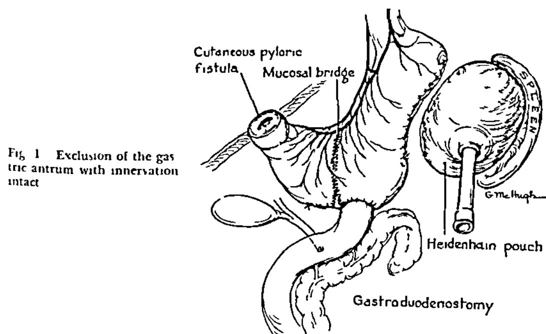



Fig. 1. Exclusion of the gastric antrum with innervation intact.

* From the Department of Surgery, the University of Chicago School of Medicine. Aided by grants from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service and from the Otho S. A. Sprague Memorial Institute.

tion of 15 units of regular insulin were measured on multiple occasions. Antrum motility was studied by introducing a small Foley balloon connected to a strain gauge and recording system through the cutaneous fistula. Observations were made under conditions of feeding, fasting and after the administration of insulin.

RESULTS

Following total exclusion of the innervated antrum the quantitative 24 hour output of hydrochloric acid remained essentially unchanged in all 4 animals. The response to insulin hypoglycemia was negative in all 4 dogs during the control period. However, after exclusion, repeatedly positive tests were seen in 2 animals and occasional positive tests were obtained in the other 2. When the dogs were fasted for a 3 day period, almost complete cessation of secretion from the Heidenhain pouch was observed, of the same order of magnitude as that which follows antrectomy (Table 1).

*Table 1. The Effect of Exclusion of the Innervated Antrum on Gastric Secretion*

| DOG NO | MEAN 24 HR SECRETION (mEq of HCl) Control | Antrum Excluded | SECRETORY RESPONSE TO INSULIN HYPOGLYCEMIA Control | Antrum Excluded | MEAN 24 HR SECRETION (mEq of HCl) EFFECT OF FASTING 3 Days Control | 3 Days Fasting |
|---|---|---|---|---|---|---|
| D 800 | 34 | 27 | – – | ++++ | | |
| D 983 | 23 | 22 | – – | ++++ | 25 | 1.2 |
| D 984 | 35 | 38 | – – | +–+– | 38 | 0.7 |
| D 992 | 25 | 21 | – – | +– | 25 | 1.9 |

Feeding a standard meal consisting of 200 gm. of horsemeat or of a proprietary dog food evoked a marked secretory response which began 15 to 30 min. after feeding and lasted for from 4 to 6 hr. The magnitude of the response was as great as that customarily observed in a Heidenhain pouch dog with intact antrum. However, the duration of the response was 2 to 3 times longer than that usually seen.

Motility studies performed during periods of fasting revealed the presence of waves of low amplitude occurring at a rate of 2 to 3/min. Fifteen to 30 min. after feeding rhythmical contractions of the antrum of much greater amplitude and increased frequency commenced and persisted during the entire secretory phase. Occasionally a brief spurt of activity was observed immediately after the dog had eaten, followed by the usual motility pattern. These were felt to represent vagally induced contractions.

Motility studies were also performed following the administration of insulin. Antral contractions were usually observed from 30 to 60 min. after the drug had been given, at the time that the hypoglycemic reaction was most marked (Fig. 2).

DISCUSSION

If its innervation be preserved, the gastric antrum continues to function despite complete exclusion from gastrointestinal continuity. Indeed, the antral phase of secretion as measured by quantitative daily secretion of gastric

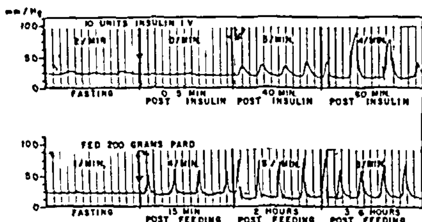



Fig 2 The effect of feeding and insulin hypoglycemia on antrum motility

juice from denervated pouches remains as great as before exclusion The nature of the preparation eliminates the possibility that regurgitated food or intestinal content is responsible for this phenomenon What then is the mechanism?

Previous investigations have suggested that distension and peristalsis of the antrum initiate the release of gastrin[3] In the studies reported here it would appear that those events which induce motility of the antrum lead to production of gastrin as demonstrated by secretion of gastric juice from the antrum phase pouch Thus feeding the animals which would be expected to induce vagal stimulation of antrum motility as well as peristalsis transmitted by way of the intact intramural autonomic reflexes evokes a secretory response while fasting with its concomitant decrease in peristaltic activity of the stomach results in a virtual cessation of antral phase secretion Insulin hypoglycemia leading to parasympathetic stimulation produces a definite although small secretory response Motility studies confirm that those phenomena which induce secretion of gastrin origin are associated with antrum motility

It is of interest that the quantitative daily secretion of these animals is little affected by total exclusion One might infer that the actual presence of food in the antrum contributes nothing to the elicitation of antrum function This inference is probably not justified since the pH of the antrums under investigation here is neutral and the inhibitory effect of acid which obtains in the normal dog is not active in these animals

## SUMMARY AND CONCLUSIONS

Total exclusion of the gastric antrum from intestinal continuity with preservation of its intrinsic and extrinsic nerve supply does not abolish or quantitatively decrease the gastric phase of secretion Those phenomena which lead to motility of the antrum are associated with a secretory response of antrum origin It is concluded that motility of the antrum produced by peristalsis is an adequate stimulus for antral phase secretion

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At a third procedure in this animal the antrum muscularis colon mucosa hybrid antrum was excised and Heidenhain pouch secretions collected

The remaining 5 animals in the study were reoperated following a suitable control period of collections from the Heidenhain pouches All muscularis was stripped and excised from the antrum of the stomach utilizing again the submucosa cleavage plane Muscularis was stripped from the mid colon in two 8 cm lengths with individual blood supply The stripped colon mucosa was excised and continuity re-established by end to end anastomosis The 2 colon muscularis strips were then sutured to the bare antrum mucosa as an anterior and a posterior patch completely covering it in an antiperistaltic fashion At all times the bare mucosa and muscular strips appeared pink and viable Secretions from the Heidenhain pouches were again collected for a suitable period

At a third procedure in these 5 animals the hybrid antrum was excised from the stomach and transplanted to the midcolon as a diverticulum Gastroduodenostomy re established gastrointestinal continuity This simulates with the antrum mucosa colon muscle hybrid antrum the antrum to colon preparation Twenty four hour quantitative secretions were again collected volume and free acid determined Two of the animals died following this procedure

In 2 of the 5 animals a fourth procedure was completed The hybrid antrum diverticulum of the colon was resected from the colon fitted with a cannula and closed The antrum cannula was brought out through a right abdominal stab wound and the colon defect was sutured closed The animal in this manner was converted to a Heidenhain pouch hybrid antrum pouch preparation It must be noted that the blood supply of this hybrid antrum during the last 2 procedures was derived entirely from the vessels supplying the transplanted colon musculature and these vessels were carefully preserved throughout

All of the animals remained healthy throughout the study periods indicated except as stated Diets were changed at no time except for the immediate postoperative periods varying from 3 to 7 days During the immediate postoperative period no pouch collections were made Penicillin was administered routinely for the first 3 days postoperatively

## RESULTS

It is apparent in D 52 that the antrum musculature is unable to release gastrin If it were the site of gastrin release its position covering a diverticulum of colon mucosa should cause a hypersecretion from the Heidenhain pouch if we can reason from the antrum to colon preparation Yet it produced no hypersecretion in fact secretion fell as though the animal had been deprived of its antrum

Because the musculature of the antrum covering a diverticulum of colon mucosa and thus in a position of maximal stimulation produced no response from the Heidenhain pouch the reverse situation was prepared Antrum mucosa was stripped of its musculature and covered with colon muscle placed antiperistaltically in an attempt to produce gastric stasis and give this preparation every opportunity to demonstrate an antrum phase of secretion Surprisingly secretions from the Heidenhain pouches in these animals also dropped to levels comparable to that produced by antrectomy It should

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# STUDIES ON THE SITE AND MECHANISM OF GASTRIN RELEASE*

C M BAUGH J BARCENA J BRAVO AND L R DRAGSTEDT

The importance of the gastric antrum as the site of release of a humoral stimulant of gastric secretion has been demonstrated and elaborated upon in a variety of ways Distention of the antrum [1] and acetylcholine[2] or liver extract applied locally to the antrum are known to release gastrin Locally applied acid topical anesthetics and systemic atropine[3] are known to inhibit the gastrin mechanism Transplantation of the antrum to the colon as a diverticulum[4] is a powerful stimulant of gastric secretion via the gastrin mechanism However the mechanism of release of this humoral agent called gastrin and its site of release in the antrum have not been demonstrated

In order to study this problem it was decided to isolate antrum muscularis from mucosa and follow quantitative 24 hr secretions from a denervated pouch (Heidenhain pouch)

## METHOD

In each of 6 healthy adult mongrel dogs a Heidenhain pouch was constructed in the usual manner and fitted with a stainless steel cannula which was brought out through a left abdominal stab wound The cannulae were fitted with football bladders and 24 hr secretions were collected quantitatively Free hydrochloric acid was determined by titration with one tenth normal sodium hydroxide using p dimethylaminoazobenzene as indicator

After a suitable control period of collections from the Heidenhain pouch one of the animals was reoperated The stomach was transected at the antro fundic junction and all antrum mucosa was stripped out utilizing the sub mucosal cleavage plane The antrum mucosa was excised in the pylorus and the open mucosa of the duodenum infolded by suture The bare antrum muscle receiving its blood supply from its attachment to the duodenum was then transplanted to cover a surgical diverticulum of the colon mucosa to form an antrum muscle-colon mucosa hybrid variation of the reported antrum to colon preparation Gastrojejunostomy reestablished gastrointestinal continuity Twenty four hour collections from the Heidenhain pouch were again followed

* From the Department of Surgery of The University of Chicago Aided by grants from the Otho S A Sprague Memorial Institute and from the Division of Research Grants and Fellowships of the National Institutes of Health U.S Public Health Service

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*Table 1 Average daily volume clinical units and mEq of free acid secreted from the Heidenhain pouch during a control period hybrid antrum period after the hybrid antrum had been transplanted to the colon, and after the animal had the antrum excised or converted into a pouch Note that D 52 is a different preparation than the others listed*

| DOG NO | HEIDENHAIN POUCH CONTROL PERIOD AVER VOL | FREE ACID | mEq | # OF COLL | HYBRID ANTRUM AVER VOL (cc) | FREE ACID | mEq | # OF COLL | HYBRID ANTRUM TRANSPLANTED TO COLON AVER VOL. (cc) | FREE ACID | mEq | # OF COLL | ANTRECTOMY OR ANTRUM POUCH AVER VOL (cc) | FREE ACID | mEq | # OF COLL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D 168 | 136 | 81 | 11 0 | 90 | 64 | 72 | 4 6 | 34 | 132 | 105 | 13 7 | 37 | 106 | 87 | 9 2 | 31 |
| D 172 | 416 | 117 | 48 7 | 55 | 68 | 81 | 5 5 | 46 | ( | | Dead | | | | | ) |
| D 202 | 672 | 133 | 89 4 | 44 | 154 | 93 | 14 3 | 30 | 473 | 119 | 56 3 | 20 | ( | Dead | | ) |
| D 206 | 161 | 117 | 18 8 | 47 | 74 | 61 | 4.5 | 32 | 199 | 110 | 21 9 | 35 | 52 | 43 | 2 2 | 31 |
| D 280 | 239 | 118 | 28.2 | 27 | 94 | 71 | 6 7 | 31 | | | | | | | | |
| D 52 | 397 | 110 | 43 7 | 39 | | | | | 72 | 15 4 | 1 1 | 44 | 80 | 72 | 5 8 | 51 |

be noted that the pouch secretions showed no tendency to rise toward the end of the collection periods in any of these animals Temporary vascular embarrassment was thus excluded as an explanation for the drop in secretions

With no activity of the gastrin mechanism evident from the antrum mucosa while remaining in its normal relation to the stomach it appeared that neither the antrum musculature nor the antrum mucosa was separately capable of releasing gastrin However doubt remained that both layers should be inactive Therefore it was decided to transplant this antrum mucosa colon muscle hybrid antrum to the colon as a diverticulum in order that the site of gastrin release be in a position of maximal stimulation

Following transplantation of the antrum mucosa colon muscle hybrid antrum to the colon an amazing increase in 21 secretions from the Heidenhain pouches occurred indicating that the gastrin mechanism was present and active at least in this location *However, it must be emphasized that* these animals did not hypersecrete with the hybrid antrum in this position This together with the fact that there was little or no evidence of antrum function with the hybrid antrum in its normal position in the stomach suggests that the surgery traumatized the antrum to such an extent that greater stimuli were required to activate the gastrin mechanism of the hybrid antrum than are normally present in a dog s stomach

A final confirmatory procedure of constructing a pouch of the hybrid antrum diverticulum was done after resecting it from the colon In the animals surviving this procedure secretions from the Heidenhain pouches again dropped much as one would expect if working with a normal antrum In this position the normal antrum is effectively non functioning and this proved to be true of the antrum mucosa-colon muscle hybrid antrum

## DISCUSSION

Four items were established at this point (a) gastrin is released from one or a combination of structures in the mucosa and submucosa (b) gastrin can be released in the absence of the antrum musculature and its contained nervous structures (c) the gastrin mechanism remains quite viable and is responsive despite these extensive surgical procedures (d) in order to maintain a normal gastrin mechanism the antrum layers must remain intact

With the gastrin mechanism thus localized to the antrum mucosa and submucosa speculation as to the cellular site of gastrin can reasonably be limited to 2 structures e g mucosal cells and/or nervous tissue such as Meissner s plexus The lack of histological differentiation of the antrum mucosa cells by present techniques allows suspicion to point more strongly toward *nervous tissue as the site of gastrin release* This is supported by the manner in which the antrum responds to acetylcholine topical anesthetics and systemic atropine The release of gastrin from nervous structures could either be direct or mediated by a local reflex arc

## SUMMARY

The procedure of making a hybrid gastric antrum either of antrum muscle and colon mucosa or antrum mucosa and colon muscle has been described Hybrid antrums prepared in this manner demonstrated that the antrum muscularis containing its nervous plexi is not the site of formation

or release of gastrin Gastrin is released from the gastric antrum mucosa and/or submucosa The suggestion is presented that gastrin is released from Meissner s plexus or other nervous structures in the antrum submucosa or mucosa either by direct stimulation or by way of a local reflex arc


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# INHIBITION OF THE INTESTINAL PHASE OF GASTRIC SECRETION BY ANTRAL INHIBITOR SUBSTANCE*

BENJAMIN D MARGOLUS AND R CAMERON HARRISON

Dragstedt and his associates demonstrated the influence of pH in the region of the antrum on gastric secretion Having shown that *the presence of* acid in the antrum depressed acid production by the body of the stomach they concluded that this action was entirely due to a reduction in gastrin production by the antrum Other workers have suggested that the antrum might secrete an inhibitor substance Previous experimental work done in our laboratory demonstrated this and further indicated that the mode of action of this substance is humoral However its site of action has not been established and its possible influence on other phases of gastric secretion has not been clarified The following experiment suggests that this antral inhibitor is also effective against the intestinal phase of gastric secretion

METHOD

Modifying a preparation described by Woodward and his associates 3 dogs were prepared with Heidenhain and total antrum pouches The whole antrum was separated from the body of the stomach excising the junctional area to ensure that no acid secreting mucosa remained in the antral pouch A stainless steel cannula was placed in the antrum and the pyloric end of the antral pouch was brought through the abdominal wall as a mucocutaneous fistula The duodenal stump was closed and gastrointestinal continuity re established by anastomosing the main stomach to the distal portion of the duodenum (Fig 1)

* From the Department of Surgery and the McEachern Laboratory University of Alberta Medical School Alberta Can

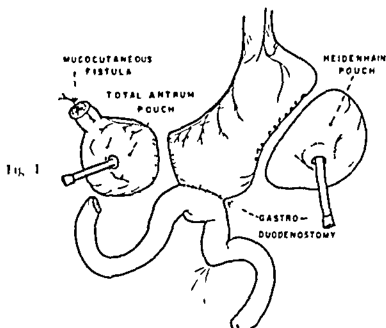



Fig. 1

After a recovery period of 3 to 4 wks during which time the dogs were trained to stand in a Pavlov frame the antral pouch secretion was tested for acid following the injection of a stimulating dose of histamine (1 mg) Invariably the secretion that followed contained no free HCl so we were confident that no acid producing mucosa had been left in the antrum

The dog in the fasting state was placed in the Pavlov frame and the antral pouch continuously irrigated with N/10 HCl After 30 min the animal was fed a standard meal consisting of 1 can of dog food and Heidenhain pouch secretion collected for 4 hr During the control experiment the ani

*Table 1*

| 4 HR HEIDENHAIN POUCH SECRETION | | ANTRUM IRRIGATED WITH 0.9% NaCl VOLUME cc | HCl m Eq /l | ANTRUM IRRIGATED WITH N/10 HCl VOLUME cc | HCl m Eq /l |
|---|---|---|---|---|---|
| Dog A Test No | 1 | 39 | 194 | 1.5 | 8 |
| | 2 | 23 | 192 | 1.0 | 4 |
| | 3 | 26 | 119 | 2.0 | 4 |
| | 4 | 21 | 195 | 0.5 | 0 |
| | 5 | 58 | 222 | 0.5 | 0 |
| Dog B Test No | 1 | 60 | 153 | 3.0 | 4 |
| | 2 | 20 | 198 | 2.5 | 0 |
| | 3 | 35 | 156 | 4.0 | 14 |
| | 4 | 10 | 72 | 1.0 | 0 |
| Dog C Test No | 1 | 34 | 138 | 4.0 | 0 |
| | 2 | 19 | 102 | 2.0 | 6 |
| | 3 | 22 | 156 | 7.0 | 6 |
| | 4 | 17 | 81 | 1.5 | 4 |
| | 5 | 10 | 100 | 5.0 | 0 |

mal again in the fasting state was placed in the Pavlov frame and the routine of feeding and collection of Heidenhain pouch secretion was repeated However on this day the irrigating solution used was normal saline Several determinations with both irrigating solutions were made in each of the 3 dogs

### RESULTS

Perfusion of the antral pouch with N/10 HCl resulted in marked diminution of Heidenhain pouch secretion The amount of acid in the secretion was markedly reduced or totally inhibited in all determinations (Table 1)

### DISCUSSION

In this preparation the Heidenhain pouch was denervated and the antrum completely separated from the remainder of the gastrointestinal tract The secretory response of the Heidenhain pouch following the ingestion of food could therefore be presumed to represent intestinal phase secretion

Since it had previously been shown that mechanical distention of the antrum stimulates gastric secretion during our control determinations normal saline was used to perfuse the antral pouch However the perfusing solutions did not appear to stimulate secretion mechanically as Heidenhain pouch secretion was not found to vary significantly with or without saline perfusion

This experiment would indicate that the antrum in an acid environment produces an inhibitor substance capable of inhibiting the intestinal phase of gastric secretion

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## THE EFFECT OF CORTISONE ON HISTAMINE STIMULATION OF GASTRIC SECRETION IN THE ADRENALECTOMIZED DOG*

BERNARD SIGEL JAMES G BASSETT AND DONALD R COOPER

With the addition of ACTH and cortisone to medical therapeutics there have been many reports of exacerbations of symptoms and complications of peptic ulcer in individuals receiving these steroids The work of Gray[1 2] indicates that an increase in gastric secretion which he has observed following administration of these hormones may be responsible for this occurrence The means by which this increase of gastric secretion is effected however is not clear Whether cortisone exerts a direct influence on parietal cells plays a permissive role in relation to other mechanisms or exerts an indi

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*From the Department of Surgery Woman's Medical College of Pennsylvania Philadelphia Pa Blood chemical determinations were performed in the laboratory of Dr David Seligson

With the technical assistance of Janet A Parker and Elsa D Kertesz

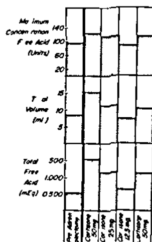
rect effect involving its action on water and electrolyte metabolism seem to be important possibilities which warrant consideration. This is a preliminary report of a study which proposes to elucidate certain actions of cortisone on gastric acid secretion in adrenalectomized dogs. In this way, the specificity of cortisone can be observed particularly in comparison to other forms of adrenal cortical replacement and also during controlled variation of other conditions.

## METHOD

Mongrel dogs weighing 20 to 25 lbs were used. Small Pavlov pouches were constructed in 4 animals and insulin testing revealed 1 of these to be innervated and 3 denervated. Histamine pouch analyses were then performed in the following manner: 1) the animals were fasted for at least 18 hr and the tests were performed in the morning; 2) the basal pouch secretions were tested and in nearly all instances were found to contain no free acid; 3) histamine diphosphate 0.5 mg was injected subcutaneously; 4) the animals were trained to stand or sit quietly while pouch collections were obtained every 15 min for 90 min; 5) the collected specimens were titrated for free and total acid. The dogs were fed a standard laboratory diet following the tests and unless certain conditions of the experiment called for it this was not supplemented or substituted. Following these base line studies single stage bilateral total adrenalectomies were performed utilizing lumbar approaches. One week after adrenalectomy the animals were again subjected to histamine pouch analyses similar to those conducted during the base line period. These tests were now performed under varying conditions of adrenal cortical replacement. At least 18 hr were allowed for each dog to be acclimatized to a new condition before testing was begun and 2 to 8 histamine tests were performed during each period. The only exception to this was when adrenal crisis was induced in less than 48 hr and histamine testing was performed earlier. Serum electrolyte and urea nitrogen determinations were performed during most of the test conditions.

## RESULTS

The total mEq of free acid secreted was markedly increased when the adrenalectomized animals were receiving 50 mg of cortisone. As the dosage




*Relationship of Cortisone Dosage to Histamine Induced Gastric Acid Secretion*

*Adrenalectomy in two Heidenhain Pouch Dogs (1B and 10B) Average*

Fig 1. The effect of cortisone dosage on histamine induced gastric acid secretion

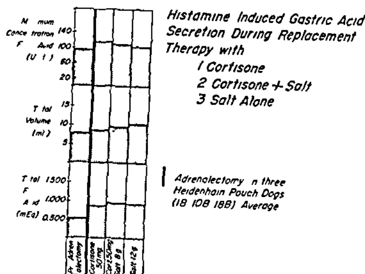



Fig 2 Comparison of cortisone to other forms of replacement therapy

was reduced the free acid produced during the testing period concomitantly decreased This was mainly a function of volume of gastric juice for the peak concentrations of free acid were only slightly affected When the cortisone was again increased there was a rise in acid secreted (Fig 1)

When cortisone 50 mg daily was administered the response to histamine was greater than before adrenalectomy Cortisone 50 mg plus 8 gm of supplementary salt had a comparable effect The results, when 12 gm of salt only were administered were essentially the same as when cortisone or cortisone plus salt was given (Fig 2)

When no substitution therapy was administered the animals developed anorexia vomiting marked weakness weight loss and bloody diarrhea Gastric secretion at this time was markedly suppressed the total mEq volume and concentration being equally affected (Fig 3)

When adrenal insufficiency was treated with 50 mg of cortisone daily but the animals maintained on a low salt diet clinical symptoms ameliorated but serum sodium and chloride remained low Histamine was capable of evoking a good acid secreting response at this time (Fig 3)

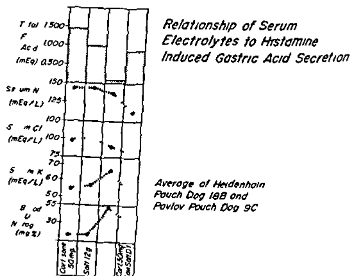



Fig 3 The effect of adrenal insufficiency on gastric acid secretion and the effect of low serum concentrations of sodium and chloride during cortisone replacement therapy

## DISCUSSION

Animals submitted to total adrenalectomy provide an excellent preparation for observing certain effects of the adrenal glands on gastric acid secretion. When an adrenalectomized animal is adequately maintained on cortisone or sodium chloride, a powerful stimulant such as histamine can evoke a good secretory response. *In fact, when large amounts of cortisone or salt are given, this response is greater than in the intact animal.* The reason for this is not made clear by this study. However, it is evident that histamine can produce its effect on gastric acid secretion in the complete absence of cortisone or for that matter the adrenal cortex itself provided the animal is kept out of acute adrenal cortical insufficiency by salt replacement. In this respect then, cortisone does not play a "permissive" role. Since only histamine has been tested, it remains for further investigation to determine if this is true for other mechanisms of gastric acid secretion. Such studies are in progress.

Serum concentrations of sodium and chloride do not seem to be the *sine qua non* for acid production by histamine, for this can occur with low concentrations of these electrolytes if cortisone is administered. It appears therefore that histamine stimulated acid secretion in the adrenalectomized *dog can occur provided that the deleterious effects of adrenal cortical in*sufficiency can be circumvented by replacement therapy.

## CONCLUSIONS

1. Following total ablation of the adrenal glands the gastric acid secreted in response to histamine in the pouch dog varies directly with the dose of cortisone administered.

2. *Dogs exhibiting signs of frank adrenal cortical insufficiency demon*strate marked reduction in both volume and concentration of gastric acid in response to histamine.

3. In the dog the adrenal glands or their products are not a prerequisite for gastric acid secretion induced by histamine.

4. It is unlikely that the effect of adrenal steroids on histamine stimulated secretion of gastric acid is secondary to their action on serum electrolyte concentrations.

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# PEPSIN IN GASTRIC PHYSIOLOGY EFFECT OF ULCER DIATHESIS ACTH HISTAMINE, ANESTHESIA OPERATION BANTHINE PILOCARPINE EPINEPHRINE AND SEDATION UPON BLOOD AND URINE PEPSIN LEVELS AND UPON GASTRIC ACIDITY*

ALBERT L MEENA J HAROLD CONN HANS NAUMANN AND JAMES D HARDY

During the past 5 to 10 years there has appeared a considerable volume of literature concerning 2 proteolytic enzymes related to gastric pepsin namely uropepsin in the urine and pepsinogen in the plasma Pepsinogen is secreted almost entirely by the chief cells of the gastric mucosa [3] and approximately 1 per cent gains access to the blood stream and is excreted by the kidneys as uropepsin [2]

The inter relationship of uropepsin plasma pepsinogen and gastric pepsin has been established by the disappearance of the enzyme from the urine after gastrectomy [1] the decreased concentration in the urine of patients with pernicious anemia [1, 2] the increased activity in the urine of patients with duodenal ulcer [1, 4] and the increased concentration in the urine of dogs after intravenous administration of pepsinogen [1]

The work of Mirsky and his associates indicated that the individual values of plasma pepsinogen were fairly constant from day to day varied little at different times of the day were not affected by the ingestion of food were increased in hypersecretory states such as peptic ulcer and were decreased in hyposecretory states such as gastric carcinoma Other investigators have repeated and expanded upon this earlier work

A major aim in the present study was to determine if there were a significant correlation between gastric acidity and the production of pepsinogen and to measure uropepsin and plasma pepsinogen in normal adult male hospital patients as opposed to duodenal ulcer patients It was also planned to determine the effect if any of various drugs and surgical procedures on the production of pepsinogen

## METHOD

A modification of the method of Anson[6] and Mirsky[1] was used to measure plasma pepsinogen in a blood sample and uropepsin in a 24 hr urine specimen The activity of the enzymes was measured by the amount of tyrosine and amino acid liberated by the protein breakdown of an hemoglobin substrate on incubation and determined colorimetrically by a phenol reaction Results are expressed in units [1, 6] Two hundred and fifty six determinations were made in 110 patients

## RESULTS

The mean 24 hr uropepsin excretion in 39 normal male adult hospital patients who had no complaints referrable to the gastrointestinal tract was

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With the technical assistance of William W Correll B S and Myra L Lowery

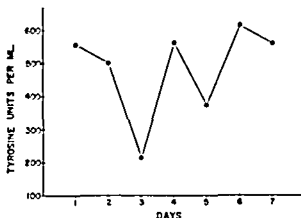



Fig 1 Note the considerable variation in levels of the same individual taken at the same hour each day

4822 ± 2446 units the mean plasma pepsinogen level was 415 ± 139 units In the present study these figures were considered to represent normal values due to modifications of technique they differ somewhat from those reported by others For instance Grey *et al* report a mean of 2300 ± 700 units of uropepsin and Mirsky *et al* report a mean of 600 units of plasma pepsinogen[8] However all groups agree that there is a wide scattering of normal values for both uropepsin and pepsinogen

In order to detect the daily variations of plasma pepsinogen blood samples were drawn at the same time of day for 7 consecutive days from one of the authors of this paper (ALM) As shown in Figure 1 there is considerable variance of the plasma pepsinogen level from day to day ranging from 215 units to 620 units These results are in disagreement with Mirsky and others who found a fairly constant day to day level of pepsinogen

To determine if there were a correlation between gastric acidity and the production of pepsinogen simultaneous measurements of basal gastric acidity plasma pepsinogen and 24 hr excretion of uropepsin were made on 22 adult male hospital patients Continuous gastric suction was maintained for 24 hr by a Levin tube and at the same time a 24 hr urine collection was made During this 24 hr period a sample of blood was drawn for the measurement of plasma pepsinogen The volume of gastric output and free and total acidity were measured No drugs were used for stimulation of gastric secretion In a statistical analysis of the data no correlation was found to exist between the amount of total or free acid and the production of pepsinogen In other words patients with a high level of gastric acidity did not necessarily have a high production of pepsinogen

Twenty-one patients who either had proven duodenal ulcers or typical ulcer symptoms were studied This group as a whole had a higher mean in both uropepsin and blood pepsinogen values than did the normals but there was a great deal of overlapping

The breakdown in the ulcer group was as follows in 5 patients with an active duodenal ulcer as proven by clinical and radiologic evidence the mean for uropepsin was about the same as for the normal group and the mean for plasma pepsinogen was slightly higher In patients who had inactive ulcers both enzyme levels were less than in the normal group In duodenal ulcer with obstruction both values were increased In the group that

*Table 1 Special Case Note the continued elevation of enzyme production even after bleeding has ceased*

| | UROPEPSIN (Units/24 hr) | BLOOD PEPSINOGEN T U /ml |
|---|---|---|
| Inactive Duodenal Ulcer (9 20 55) | 5 837 | 232 |
| Acute Bleeding Duodenal Ulcer (10 19 55) | 10 000 | 419 |
| Six Weeks After Bleeding (12 1 55) | 10 33_ | 419 |

had ulcer symptoms but a negative gastrointestinal series the means uropepsin level was increased, but the plasma pepsinogen was slightly below normal In 3 patients with actively bleeding duodenal ulcers both values were increased

**Actively Bleeding Ulcer** Much work is being done with the enzymes to aid the differential diagnosis of hematemesis It has been found by other groups that the uropepsin excretion is increased in bleeding duodenal ulcers but decreased in the presence of esophageal varices One of our patients with a previously proven duodenal ulcer was studied while he was asymptomatic during an active bleeding phase and 6 weeks after cessation of bleeding As shown in Table 1, the uropepsin and blood pepsinogen levels progressed from normal values while he was free of symptoms to high values during active bleeding which persisted long after convalescence This finding has also been observed by other investigators [8]

**Effect of Operative Stress** Preoperative and postoperative levels of pepsinogen were studied in 8 patients undergoing various types of surgical procedures (Fig 2) In several patients there was an increase in enzyme activity immediately following surgery with a gradual decrease over the next 4 or 5 days but this pattern was not uniform These observations may be explained

EFFECT OF SURGERY ON THE BLOOD PEPSINOGEN LEVEL

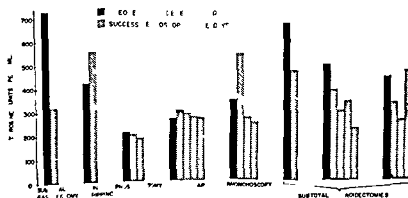



Fig 2 The levels are expressed in Tyrosine units/ml There was no consistent variation following operation

on the basis of Mirsky's assumption that stress stimulates pepsinogen production[5]

**Effect of Cortisone, Ephedrine, Pilocarpine, Thorazine, and Probanthine** Twenty-one patients were studied to determine if certain drugs would affect the output of pepsinogen and uropepsin Control enzyme levels were determined the drug administered for 3 days and the levels again determined In general both the uropepsin and pepsinogen levels changed proportionately Cortisone (7 patients) and ephedrine (6 patients) caused a mean decrease in the enzymes while pilocarpine (5 patients) and thorazine (2 patients) caused an increase The results with probanthine (1 patient) were equivocal Where variations occurred the uropepsin and pepsinogen levels changed proportionately The number of cases in each group does not yet permit statistical evaluation

## SUMMARY AND CONCLUSIONS

A wide range of uropepsin and plasma pepsinogen values in normal subjects was found Moreover in a given individual there was a considerable fluctuation from day to day Correlation between uropepsin and plasma pepsinogen values and the gastric acidity was poor The results in ulcer patients were not constant although high values were found in peptic ulcer with obstruction or bleeding and low values after gastrectomy Following surgical trauma there were rather wide fluctuations of values with a tendency toward a decrease in pepsinogen production on successive postoperative days Some drugs caused a marked effect partly stimulating partly inhibiting but here again no uniformity was observed

*It is concluded* that the plasma pepsinogen level (more conveniently determined than uropepsin) is of limited value as a diagnostic adjunct to conventional procedures in the diagnosis of conditions associated with hyper or hyposecretion of the gastric mucosa While not closely related there does exist a rough parallelism between plasma pepsinogen and gastric acidity at the extremes of the scale that is in patients with marked hyperacidity (complications of peptic ulcer) the plasma pepsinogen level is usually elevated and in patients with low or absent acidity (gastric resection cancer and pernicious anemia) the pepsinogen level is usually diminished In cases where examination by barium study gastric analysis or gastroscopy is not helpful as in hemorrhage the plasma pepsinogen level may be of assistance in differentiating between esophageal and gastric lesions

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# INCREASE IN DIGESTIVE ACTION OF HUMAN GASTRIC JUICE FOLLOWING MAJOR SURGICAL PROCEDURES*

JOHN L. PERRY, JR., EARL C. YONEHIRO, PO MYAT YA, HARLAN D. ROOT AND OWEN H. WANGENSTEEN

Ulcers of the upper gastrointestinal tract first appearing after surgical procedures are serious complications as hemorrhage and perforation often accompany such lesions and the mortality attending them is high.[1] The data to be reported here indicate that increases in the concentration of pepsin occur in the gastric juice of patients in the early postoperative period and that these increments are such as to be associated with a digestive capacity of the juice for living tissues such as that seen with gastric secretion obtained from known duodenal ulcer patients. In addition, an increase in the hydrochloric acid content of the gastric juice, but to a less significant degree, occurs. This increase in the concentration of pepsin and possibly of acid would seem to be of fundamental importance in the genesis of those ulcers occurring in the post surgical period.

## METHOD

Gastric secretions were collected from fasting patients by inlying gastric tube attached to suction for the 8 hr. period from 11 p.m. to 7 a.m. Only patients who were to undergo major extragastric surgical procedures were chosen. A collection was made prior to operation and again within the first 96 hr. following the operative procedure. Gastric secretions were collected over ice and were analyzed immediately or stored below 0°C. until used. The volume of each specimen was measured and a sample removed for determination of pH, HCl and pepsin. Free HCl was determined by titration with .1 N NAOH. pH was measured by the Northrup and Leeds glass electrode and pepsin was determined by the hemoglobin substrate method of Anson[6] and expressed as tyrosine units per ml. of gastric juice. The remaining gastric juice was adjusted to pH 1.6–1.7 with hydrochloric acid to provide an optimum medium for peptic activity and used to perfuse the esoph

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*From the Department of Surgery, University of Minnesota Medical School, Minneapolis 14, Minnesota. Supported by U.S.P.H.S. #RG 1028 (C9), Malignant Disease Research Fund, Arthur and Stella Sanford Fund, Austen S. and Anne T. Cargill Fund for Surgical Research.



Fig 1

agus of a living anesthetized cat for a period of 2 hr as previously described[3] The animal was then sacrificed and the esophagus examined grossly and microscopically The degree of digestion of the esophagus produced was graded as follows *Grade O* No injury or only hyperemia of the esophageal mucosa *Grade 1* Erosion of the mucosal epithelium *Grade 2* Digestion into the submucosa *Grade 3* Digestion into the muscular layers of the esophagus *Grade 4* Impending perforation *Grade 5* Actual perforation during the perfusion period Figure 1 shows minimal (Grade 0) and maximal (Grade 5) degrees of esophageal digestion produced Statistical significance of mean values obtained was tested by use of the t test[4]

RESULTS

For purposes of comparison the results obtained by studying in the same manner the gastric secretions of a group of control patients duodenal ulcer patients and a large group of postoperative patients are shown in Tables 1 and 2[3] The pepsin concentration was significantly higher in both the duodenal ulcer group and the postoperative group as compared with controls

*Table 1 8 Hour Night Secretion*

| | TOTAL NO PATIENTS | FREE HCl MEAN | PEPSIN MEAN | pH (2 0 OR BELOW) |
|---|---|---|---|---|
| Control | 53 | 2 87 mEq | 1 0 u/cc | 16% |
| Postoperative | 61 | 7 60 mEq | 1 4 u/cc | 29% |
| Duodenal Ulcer | 18 | 20 90 mEq | 1 7 u/cc | 53% |

*Table 2 Perfusion of Cat Esophagus with Human Gastric Juice pH 1 6–1 7*

| | TOTAL NUMBER OF PATIENTS | GRADE OF DIGESTION 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|
| Control | 32 | 5 | 19 | 5 | 1 | 1 | 1 |
| Postoperative | 54 | 4 | 22 | 14 | 3 | 1 | 10 |
| Duodenal Ulcer | 18 | 1 | 3 | 1 | 3 | 5 | 5 |

Free acid concentration was significantly greater and the mean pH significantly lower in the duodenal ulcer group as well but not in the postoperative group as compared with controls

Table 2 shows the digestion of the cat esophagus produced by the gastric secretions of patients in these 3 groups The necrotizing effects on the cat esophagus of the gastric secretions of both duodenal ulcer patients and patients in the postoperative period were significantly greater than that produced by the gastric secretions obtained from control patients Only 10 per cent of specimens obtained from control patients caused grades 3 to 5 digestion of the cat esophagus, while 72 per cent of specimens from known duodenal ulcer patients and 26 per cent of specimens from patients in the early postoperative period caused these severe degrees of esophageal damage

Gastric secretions collected from 25 patients both before and after operation were available for analysis and comparison Tables 3 and 4 show mean values obtained for pepsin free HCl pH and volume preoperatively and after operation The gastric secretions of 13 of these patients had no free hydrochloric acid present before surgery (Table 3) Pepsin values in this group were generally low and pH values reflected the absence of free acid Following operation mean values for pepsin and free acid were higher but the differences are not statistically significant Mean pH and volume were also not significantly different from those preoperatively In 12 patients free

*Table 3 8 Hour Night Secretion Patients with No Free Hydrochloric Acid in Gastric Juice Before Operation (13 Patients)*

| | PREOPERATIVE | | POSTOPERATIVE | | |
|---|---|---|---|---|---|
| | MEAN | RANGE | MEAN | RANGE | |
| Pepsin | 49 u/cc | 1–1 8 u/cc | 1 0 u/cc | 1 2.5 u/cc | 9 increased<br>2 decreased<br>2 unchanged |
| Free HCl | 0 | 0 | 5.59 mEq | 0 41 47 mEq | 4 of 13 had free HCl after operation |
| pH | 4 8<br>(0 patients | 2 7 5 5<br>pH 2 0 or lower) | 4 3<br>(2 patients | 1.5 6 7<br>pH 2 0 or lower) | |
| Volume | 306 cc | 10 800 cc | 282 cc | 60 740 cc | |

*Table 4 8 Hour Night Secretion Patients with Free Hydrochloric Acid in Gastric Juice Before Operation (12 Patients)*

| | PREOPERATIVE | | POSTOPERATIVE | | |
|---|---|---|---|---|---|
| | MEAN | RANGE | MEAN | RANGE | |
| Pepsin | 1 87 u/cc | 3 3 7 u/cc. | 2 8 u/cc | 1 5 0 u/cc | 10 increased<br>2 decreased |
| Free HCl | 9.37 mEq | .25 19 05 mEq | 14 48 mEq | 0 40 80 mEq | 8 increased<br>4 decreased |
| pH | 2 1<br>(6 patients | 1 4 3 0<br>pH 2 0 or lower) | 2.5<br>(6 patients | 1.5 5.3<br>pH 2 0 or lower) | |
| Volume | 250 cc | 10 635 cc | 415 cc | 60 1090 cc | |

hydrochloric acid was present in the gastric juice obtained before operation. Mean values obtained before and after surgery for pepsin, free acid, pH and volume are shown in Table 4. Pepsin values were generally higher before operation than in the group with no free acid and tended to parallel the amount of free acid present. Following surgery pepsin concentrations rose to even higher values and the increase over preoperative values is statistically significant. The mean value for free acid after operation is higher but the difference in means as compared with the preoperative value is not significant. Mean pH and volume values were not significantly different in the postoperative period as compared with preoperative controls. Figures 2 and 3 show graphically the changes in pepsin concentration occurring following operation in the 2 groups. Figure 2 indicates that in the group with no free acid initially several of the patients show no change in pepsin concentration after operation and in 2 an actual decrease in concentration occurred. In the remainder increase in concentration occurred. In the group with free acid (Fig. 3) the tendency for higher pepsin values initially and greater increases in pepsin after surgery is shown. Two of 12 of these patients showed a decrease in pepsin concentration after operation and in the remainder a definite increase occurred.

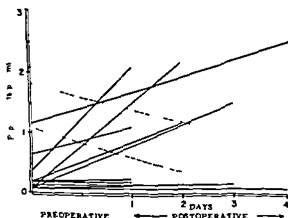
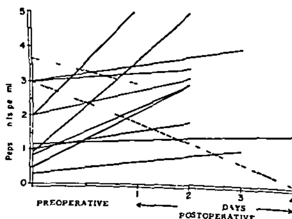



Fig. 2

CHANGES IN CONCENTRATION OF GASTRIC PEPSIN FOLLOWING OPERATION. PATIENTS WITH NO FREE HYDROCHLORIC ACID IN THE GASTRIC JUICE INITIALLY

Fig. 3




CHANGES IN THE CONCENTRATION OF GASTRIC PEPSIN FOLLOWING OPERATION. PATIENTS WITH FREE HYDROCHLORIC ACID IN THE GASTRIC JUICE INITIALLY

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| Volume | 250 cc. | 10 635 cc | 415 cc. | 60 1090 cc | |

# THE SECRETORY EFFECT OF GASTRIC TRANSECTION*

C I MOUNTAIN J H LANDOR J D McCARTHY P V HARPER
AND L. R DRAGSTEDT

The occasional failure of transabdominal supradiaphragmatic vagotomy in ulcer therapy is usually due to incomplete interruption of the nerves as shown by physiologic tests Dragstedt and Camp[1] stated that incomplete vagotomy exists in approximately 5 per cent to 10 per cent of cases Oberhelman and Dragstedt[2] reported on a series of 187 patients treated by vagotomy and gastroenterostomy of which 28 subsequently developed stomal ulcers Of these 28 recurrent ulcer cases 11 had some intact vagal fibers as evidenced by a positive gastric secretory response to insulin hypoglycemia This failure to attain a complete vagotomy in a small percentage of cases may be explained by the inconstant arrangement of vagal fibers along the lower esophagus[3] As it was felt that a more certain interruption might be accomplished by a high transection of the stomach with re anastomosis the effect of this procedure on gastric acid secretion was investigated

## METHOD

Total isolated stomach pouches were prepared by the abdominal route in 8 female mongrel dogs with restoration of gastrointestinal continuity by end to-end gastro-duodenostomy using the method described by Dragstedt *et al*[4] Stainless steel cannulae were placed in the isolated stomach just proximal to the antral fundic junction on the greater curvature side being brought to the outside through a stab wound in the abdominal wall Seven of these pouches had good vagal innervation as evidenced by consistently positive responses to insulin hypoglycemia (10 units crystalline insulin intravenously in fasting state) on repeated testing The eighth dog (D 212) had such poor response that it was considered essentially vagally denervated After recovery a standard diet of 600 gm horsemeat 500 cc milk and 800 cc water was given daily with electrolyte balance being maintained by the addition of sodium chloride When the animals were consuming the full diet rubber football bladders were attached to the cannulae by means of metal adapters and the gastric secretions were collected and measured every 24 hours A 1 cc aliquot of each 24 hr specimen was titrated for its free acid content using 0 1 N NaOH with dimethylaminoazobenzene (Toepfer s reagent) as the indicator Daily output of HCl in milliequivalents was then calculated by multiplying the volume secreted by the acidity in clinical units

After a control period varying from 48 to 134 days the upper part of the stomach pouch was completely transected along with all vessels and nerves This line of transection was carried medially across the lesser omentum After ligating the vessels with silk the 2 cut edges were anastomosed in their original position using 2 layers of #00 chromic cat gut In half of the animals (D 212 225 859 905) the transection was made across a line separating the upper one fifth from the lower four fifths of the stomach In the remaining

*From the Department of Surgery of The University of Chicago Aided by grants from the Division of Research Grants and Fellowship of the National Institutes of Health United States Public Health Service and the Douglas Smith Foundation for Medical Research of The University of Chicago

*Table 5 Perfusion of Cat Esophagus with Human Gastric Juice pH 1 6–1 7*

| | NUMBER OF PATIENTS | DEGREE OF DIGESTION | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| Preoperative | 17 | 3 | 11 | 2 | 1 | | |
| Postoperative | 20 | | 9 | 4 | 1 | 1 | 5 |

When perfusion of the cat esophagus to measure the digesting effect of the gastric juice on living tissue was carried out using specimens obtained preoperatively and postoperatively the results shown in Table 5 were obtained Significantly greater degrees of digestion were produced by the gastric secretion obtained in the postoperative period than by those specimens obtained preoperatively Both the gastric secretions from patients with free hydrochloric acid present before operation and those with no free acid showed statistically significant increases in esophageal digestion with gastric juice obtained postoperatively

## DISCUSSION

Gray and associates[6, 7] have demonstrated increases in gastric acid and pepsin and increased excretion of uropepsin to levels seen in duodenal ulcer patients in association with acute stress situations and have produced similar changes by the administration of ACTH or cortisone to patients and animals Although significant increases in HCl in the gastric juice of postoperative patients could not be demonstrated in our studies many specimens had sufficient free acid present to maintain the pH at 2 0 or below a figure well in the activity range for pepsin and it is in this group with higher acid values that the greatest postoperative increases in gastric pepsin occur These changes in the direction of those observed for gastric secretions obtained from duodenal ulcer patients offer in part at least an explanation for the mechanism of occurrence of postoperative ulcers

## SUMMARY

There occurs a significant increase in the concentration of pepsin in the gastric juice of many patients following operation Increases in acid concentration are less definite Associated with this change is an augmented capacity of the juice to digest living tissue This increased peptic activity of the gastric juice is probably of fundamental importance in the genesis of postoperative ulcers

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*Table 1 Effect of High Gastric Transection and Subsequent Bilateral Transthoracic Vagotomy on Gastric Secretions in Dogs with Total Stomach Pouches*

| | DOG NO | INSULIN TEST | TOTAL GASTRIC POUCH NO OF 24 HR COLLECTIONS | MEAN VOL | mEq FREE HCl±SD | FOLLOWING TRANSECTION NO OF 24 HR COLLECTIONS | MEAN VOL | mEq FREE HCl±SD | PER CENT DECREASE IN ACID SECRETED | AFTER TRANSTHORACIC VAGOTOMY NO OF 24 HR COLLECTIONS | MEAN VOL | mEq FREE HCl±SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TRANSECTED 2 CM FROM CARDIA | 21[illegible] | ± | 64 | 366 | 35 3±13 2 | 64 | 27[illegible] | 24 4±12 9 | 23 | 78 | 101 | 30 7±11 8 |
| | 22[illegible] | ++++ | 18 | 724 | 38 1±21 5 | 61 | 31[illegible] | 25 2±10 5 | 70 | 74 | 230 | 16± 8 4 |
| | 859 | ++++ | 134 | 316 | 30 0± 9 6 | 126 | 220 | 12 1± 5 8 | 60 | 68 | 323 | 25 9±10 3 |
| | 905 | ++++ | 89 | 356 | 31 7± 7 9 | 113 | 241 | 12 5± 5 6 | 61 | 46 | 334 | 17 8± 6 6 |
| TRANSECTED 1/3 — 4 5 | 218 | ++++ | 54 | 515 | 51 6±17 9 | 46 | 116 | 10 2± 1 0 | 81 | 57 | 174 | 11 7± 4 9 |
| | 283 | +++ | 53 | 369 | 31 3± 7 0 | 15 | 109 | 16 1± 2 6 | 53 | 30 | 200 | 16 0± 5 0 |
| | 613 | ++ | 50 | 280 | 29 7± 8 5 | 89 | 200 | 16 9± 5 5 | 43 | 63 | 188 | 16 0± 4 5 |
| | 719 | ++++ | 49 | 325 | 34 3±12 0 | 56 | 236 | 16 8± 2 0 | 51 | 48 | 196 | 14 1± 3 9 |

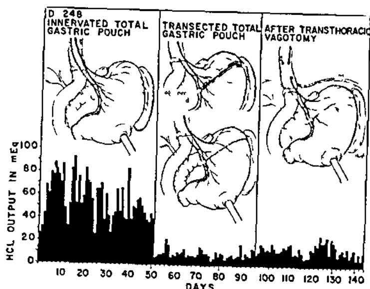



Fig. 1 The secretory effect of gastric transection

animals (D 248 283 643 719) the line of transection was approximately 2 cm from the cardia so that an extremely small amount of vagally innervated gastric mucosa remained Insulin tests conducted after transection were consistently negative except for D 905 which exhibited a weakly positive delayed response Daily secretions were again collected for periods of 45 days to 126 days following which all animals were subjected to bilateral transthoracic vagotomy Following this third operative procedure daily secretions were again collected for 30 to 78 days and the experiment was concluded All insulin tests made following this procedure were negative All of the animals remained in excellent health throughout the experimental period

## RESULTS

The effect of gastric transection and subsequent transthoracic vagotomy on the daily 24 hr secretion of gastric juice in the 8 dogs studied is summarized in Table 1 All of the animals showed a highly consistent secretory response to gastric transection with an average reduction in the daily output of free hydrochloric acid of 55 per cent In dog 212 apparently most of the vagal fibers were divided during construction of the pouch as there was no clear cut secretory response to insulin hypoglycemia This animal had the least decrease in free acid (22 per cent) following gastric transection Dog 643 had a moderate response to insulin hypoglycemia and similarly had less of a secretory decrease (43 per cent) The remaining animals exhibited a consistently strong secretory response to the insulin test and were thought to have good vagal innervation of their pouches

Considering this group of 6 dogs alone following transection there was a 62 per cent decrease in the 24 hr output of free acid ranging from 18 mEq to 58 mEq In general transection 2 cm from the cardia appears to have a slightly greater depressant effect on acid secretion but by analysis of variance this difference is not statistically significant due to the high inter dog variability Following gastric transection insulin tests were negative in all animals except for D 905 who demonstrated a delayed weakly positive secretory response Following bilateral transthoracic vagotomy none of the animals demonstrated a statistically significant further reduction in the

tion in the upper innervated portion of the stomach at the time of transthoracic vagotomy may have masked the increase in secretion resulting from denervation of the small bowel with its assumed effect on increasing the intestinal phase The efficacy of transection in reducing gastric secretions in this study seems correlated with the degree of initial vagal innervation although quantitative studies were not made to determine this

## SUMMARY AND CONCLUSIONS

Transection of the dog s stomach effectively interrupts vagal fibers as evidenced by a negative secretory response to insulin hypoglycemia The results of this study we feel indicate that high gastric transection is as effective as transthoracic vagotomy in reducing acid secretion in the isolated stomach of the dog While it is not suggested that this procedure be substituted for vagotomy in the routine clinical case it might well be a useful alternate to gastrectomy in patients with incomplete vagotomy and recurrent ulcer who require surgery The technical feasibility of this procedure in patients has been demonstrated in the management of bleeding gastric varices

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secretion of free acid However, in 2 (D 859 905) of the 3 animals in which a very high transection was carried out transthoracic vagotomy resulted in an increase in the secretion of HCl Insulin tests following this third procedure were once more negative except for D 905 which again demonstrated a delayed weak response

## DISCUSSION

The experiments reported were undertaken to determine the efficacy of vagotomy achieved by high transection of the stomach We reasoned that such a procedure if it proved effective would offer a physiologic surgical approach of low mortality and morbidity to the patient who developed a stomal ulcer and in whom intact vagal fibers could be demonstrated by physiologic tests following transabdominal supra diaphragmatic vagotomy The experimental data contained herein suggests that high gastric transection *is as effective as transthoracic vagotomy in reducing acid secretion in* the isolated stomach of the dog

Transection of the esophagus might appear to be a more effective procedure as it would result in division of vagal fibers supplying the entire mucosa but this procedure entails the technically more demanding feat of esophageal anastomosis with the attendant risk of anastomotic leak and resultant mediastinitis It might be argued that in contrast to the conventional method of resecting a small portion of the vagal trunks gastric transection with re approximation of the gastric walls might provide an ideal situation for regeneration of vagal fibers This seems unlikely in view of the findings of Dragstedt Woodward, and Camp[5] who showed that simple crushing of the vagi without disturbing their continuity caused a profound decrease in gastric secretion in dogs which persisted for as long as three years

A modification of this procedure has been used by Tanner[6] and by Allen and Head[7] for the treatment of esophageal varices which had bled Neither series is large enough nor has been followed long enough to permit definite conclusions but there were no subsequent episodes of bleeding in any of the patients followed by these authors As a surgical treatment of esophageal varices the operation was introduced primarily as a method of *interrupting* the collateral circulation between the portal system and the esophageal veins but it possesses the added advantage of reducing the fasting gastric acidity thus decreasing the likelihood that erosion of vein walls by the corrosive action of gastric juice will occur

In our present experiment the fact that transthoracic vagotomy increased the secretion in 2 of the animals previously subjected to transection of the fundus is a rather astonishing and as yet unexplained phenomenon One possible explanation may be related to the fact that as shown by Ivy Lim and McCarthy[8] and by Gregory and Ivy[9] the intestinal phase of gastric secretion in dogs is more pronounced when food does not pass rapidly through the intestine Transthoracic vagotomy may by depriving the small bowel of its extrinsic parasympathetic innervation cause disturbances of intestinal motility which result in prolonged transit time with a resultant increase in the intestinal phase of gastric secretion That this phenomenon was apparent in only two animals can be explained by assuming that transthoracic vagotomy had little direct effect on gastric secretion in these two However in the other animals the abolition of the cephalic phase of secre

of the stomach in the same operation. A stainless steel cannula was placed in the pouch for the quantitative collection of gastric juice. In 2 animals a Heidenhain pouch was made as the first procedure in the usual way and after a suitable control period during which gastric juice was collected, a total pancreatectomy was performed and the observations repeated. All animals were operated upon under general anesthesia and sterile technique. Quantitative 24 hr collections of gastric juice were secured for long periods of time in all animals. The gastric juice was titrated against 0.1 normal solution of sodium hydroxide using Topfer's reagent as the indicator. The clinical units were multiplied by the volume expressed in liters and the product represents the excretion of hydrochloric acid in mEq. Postmortem examinations were made in all cases. Sections of the liver were taken and a careful inspection of the gastrointestinal tract was done. All animals were fed a diet of horse meat, bread, milk, sugar and raw pancreas. Insulin was used as required. 3 to 6 gm of lipocaic were given orally each day.

The results are summarized in Tables 1 and 2. One of the dogs died of pneumonia 7 days after the operation and the data from this animal are excluded. Dog D 139, in which a Heidenhain pouch and pancreatectomy were performed at the same operation, survived for 58 days, dying eventually of pneumonia. Thirty eight 24 hr collections of gastric secretion were obtained in this animal and the average daily output of hydrochloric acid was 55.5 mEq. Dog D 158 survived for 57 days and no explanation for death was found at autopsy except profound cachexia. Twenty 24 hr collections of gastric juice were secured in this animal and the average daily output of hydrochloric acid was 38.9 mEq. In both of these animals the daily output of acid increased progressively from the time of operation until shortly before the animal died. In dog D 180, 64 daily collections of gastric juice from the Heidenhain pouch were made before pancreatectomy was performed. The average daily output of acid was 55.1 mEq. Seventeen daily collections of gastric juice were made after the pancreatectomy and the average output of hydrochloric acid increased to 75.4 mEq. In the second animal, D 181, 65

*Table 1 Gastric Secretion from Heidenhain Pouch in Dogs with Total Pancreatectomy*

| DOG NO | NO OF 24 HR COLLECTIONS OF GASTRIC JUICE | DAILY OUTPUT OF HCl IN mEq |
|---|---|---|
| D 139 | 38 | 55.5 ± 13.4 |
| D 158 | 20 | 38.9 ± 4.8 |

*Table 2 The Effect of Pancreatectomy on Gastric Secretion from Heidenhain Pouch*

| DOG NO | AVERAGE DAILY OUTPUT OF HCl IN mEq — BEFORE PANCREATECTOMY: NO OF 24 HR COLLECTIONS | BEFORE PANCREATECTOMY: HCl | AFTER PANCREATECTOMY: NO OF 24 HR COLLECTIONS | AFTER PANCREATECTOMY: HCl |
|---|---|---|---|---|
| D 180 | 64 | 55.1 ± 2.4 | 17 | 75.4 ± 11.4 |
| D 181 | 65 | 68.9 ± 28.4 | 18 | 65.1 ± 26.6 |

# EFFECTS OF TOTAL PANCREATECTOMY ON GASTRIC SECRETION*

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CLIFTON F MOUNTAIN AND LESTER R DRAGSTEDT

There are several observations suggesting a relationship between pancreatic function, gastric secretion and peptic ulcer formation Since the original paper of Mann and Williamson[1] attention has been called to the protective effect of external pancreatic secretion and of bile in the experimental production of peptic ulcer This beneficial effect has been attributed to the neutralizing properties of the alkaline secretions on the hydrochloric acid of the gastric juice Owens[2] has called attention to the clinical importance of this neutralizing property in patients who have had a pancreatoduodenectomy

Other observations have suggested the possible existence of humoral pancreatic factors which might produce or facilitate the production of peptic ulcers Dragstedt[3] in 1942 reported that whereas the incidence of duodenal ulcer in dogs with total external pancreatic fistulae approximates 100 per cent only 1 per cent to 3 per cent of dogs developed duodenal ulcers following total pancreatectomy In both cases, pancreatic juice was absent from the gastrointestinal tract In dogs with ligated pancreatic ducts and atrophy of the pancreatic parenchyma duodenal ulcers appeared in 29 per cent Here again, pancreatic juice is absent from the gastrointestinal tract Poth[4] studied the frequency and severity of ulcer formation in pancreatectomized and total pancreatic fistulae dogs when treated with daily injections of histamine in beeswax and concluded that the presence of external pancreatic secretion was a very important factor in the prevention of this type of experimental ulcer The same author has suggested the possibility that insulin or some hyperglycemic factor in the pancreas may play a very important role in ulcer production[5, 6]

Forty and Barret of England[7] and Zollinger and Ellison in this country[8] have called attention to patients with recurrent atypical progressive peptic ulcers associated with hyperacidity and hypersecretion which were not controlled by vagotomy and extensive gastric resection These patients had islet cell tumors of the pancreas but in no case were there symptoms suggestive of or laboratory results compatible with hyperinsulinism The hypersecretion of gastric juice was attributed to the presence of the islet cell tumor The finding of a hyperglycemic factor in the serum of one of these patients suggested the possibility that these tumors may produce this factor and in turn stimulate gastric secretion

The present experiments were devised to study the effect of total pancreatectomy on the secretions of gastric juice from Heidenhain pouches in dogs

## METHOD

In the first group of 3 female mongrel dogs a total pancreatectomy was performed and a Heidenhain pouch constructed from the greater curvature

*From the Department of Surgery of The University of Chicago Aided by grants from the Otho S A Sprague Memorial Institute and from the Division of Research Grants and Fellowships of the National Institutes of Health United States Public Health Service

# THE EFFECT OF ANASTOMOSIS OF THE PANCREATIC DUCT TO THE GASTRIC ANTRUM ON THE PRODUCTION OF EXPERIMENTAL PEPTIC ULCER*

JOHN R BROOKS

It is a clinical fact that peptic ulceration of the second portion of the duodenum just distal to the entrance of bile and pancreatic secretions is rare Experimental data suggest that the high alkalinity of these secretions is capable of buffering gastric acidity and thereby prevents mucosal ulceration The Mann Williamson[1] preparation by diverting these secretions removes this buffering protection and ulceration occurs The work of Poth[2] Dragstedt[3] and others revealed that duodenal ulceration is apt to occur in dogs following the experimental production of a pancreatic fistula or following ligation of the pancreatic ducts but that total pancreatectomy (removal of internal as well as external secretion) does not have the same ulcerogenic action This work suggests that there is some acid stimulating factor, probably endocrine within the pancreas with which the alkalinity of external pancreatic secretion competes

Dragstedt s work with antral and Heidenhain pouches[3 4] has shown that reflux of alkaline secretions or puddling of substances of high pH in the antrum of the stomach acts as a stimulus to gastric hydrochloric acid production and that substances of low pH have a reverse effect

Although our knowledge of the role of external and internal pancreatic secretion in the control of gastric secretion is still not clear it appears that the pancreas may have, indirectly or directly both stimulating and buffering actions

It is the purpose of this experimental work now in its early stages to further investigate the part played by *external* pancreatic secretion and bile in the prevention of ulcer formation and to attempt to clarify the effect of reflux of alkaline secretions (pancreatic or biliary) into the antrum

## METHOD

Twenty four adult mongrel dogs (male and female) have been operated upon The animals were anesthetized with nembutal intravenously Mann Williamson procedures were carried out in all cases The duodeno ileal anastomosis was placed within 24 in of the cecum in each case Postoperatively the animals were fed Ken L Ration and milk without bile salt or pancreatic substitution The animals were re explored in 4 to 8 wks In some instances ulceration had not occurred and a later re exploration was necessary to await ulcer formation All ulcers were proven by laparotomy and duodenotomy

## RESULTS

Four animals died at varying times (2 to 3 wks) in the postoperative period 2 of distemper and 2 of causes unexplainable at postmortem study All of these animals were thin and emaciated at death but none had evidence of ulcer formation

Two animals developed Mann Williamson ulcers which perforated and resulted in death before a second operation could be carried out

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daily collections of gastric juice were secured before pancreatectomy The average daily output of hydrochloric acid was 68 9 mEq After pancreatectomy 18 daily collections of gastric juice were secured and the average daily output of hydrochloric acid was 65 1 mEq or approximately the same as before the pancreatectomy

## DISCUSSION

It is clear from these experiments that complete removal of the pancreas in dogs does not abolish or significantly reduce the secretion of gastric juice from the Heidenhain pouch Total pancreatectomy thus does not abolish the humoral phase of gastric secretion The marked increase in secretion observed in dog D 180 after pancreatectomy may be significant

## CONCLUSIONS

1 Total pancreatectomy does not abolish the secretion of gastric juice from a Heidenhain pouch in the dog

2 Total pancreatectomy may produce a significant increase in the secretion of gastric juice from the Heidenhain pouch

3 The antrum phase of gastric secretion is little affected by pancreatic function

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which time the injections were discontinued This dog is still alive and healthy 8 mo after pancreatic duct anastomosis One other dog was given histamine 1 mon following a Mann Williamson preparation No pancreatic duct anastomosis was done This animal died in 3 days of perforation and necrosis of the first portion of the jejunum just distal to the gastrojejunostomy

**C Common Duct Anastomosis to the Antrum (Choledochoantrostomy)** Two animals with Mann Williamson ulcers were operated upon and anastomosis of the common duct carried out in a manner identical to that with the pancreatic duct The pancreatic secretions continued to drain into the lower ileum One of these animals showed complete ulcer disappearance 4 wks after anastomosis One showed significant decrease in ulcer size to signify healing

One animal had a Mann Williamson procedure and choledochoantrostomy carried out at one sitting This animal had not developed the typical Mann Williamson ulcer at the time of laparotomy 8 wks later

## DISCUSSION AND CONCLUSIONS

This investigative work is in its early stages and for this reason many of its ramifications have not yet been explored

Anastomosis of the major pancreatic duct to the antrum of the stomach above a Mann Williamson ulcer results in healing of this ulcer so long as the anastomosis remains patent The constant stimulus to gastric secretion of the presence of highly alkaline pancreatic secretion in the antrum is apparently not sufficient to overcome the strong acid neutralizing action of pancreatic bicarbonate and for this reason healing of the ulcer occurs

The number of animals in the choledochoantrostomy series is small but the results suggest that bile delivered to the gastric antrum above a Mann Williamson ulcer can also produce healing of such an ulcer

Healing of these experimental ulcers following bile and pancreatic duct anastomosis seems most easily explainable on the basis of constancy of alkalinity In the antrum as noted above such alkalinity may have two effects one to set off the antral phase of gastric secretion the other to act as a hydrochloric acid buffer The addition of histamine to the experimental preparation upsets the balance and results in greater hydrochloric acid production and ulcer formation Experiments are now under way to investigate the effect upon hydrochloric acid secretion from a Heidenhain pouch of anastomosing the pancreatic duct to a previously constructed antral pouch Experimental evidence suggests that basal Heidenhain pouch secretory levels will rise as alkaline pancreatic secretion enters the antral pouch

Clinically we have occasion to take advantage of this constancy of alkalinity in patients with duodenal ulcer disease Medical regimens for ulcer have been directed for years at the maintenance of duodenal neutrality If that portion of the duodenum just distal to the entrance of alkaline pancreatic and biliary secretions is excessively well protected against acid peptic digestion then there would be clinical reason to consider anastomosing the gastric stump to the second portion of the duodenum just distal to the ampulla of Vater following gastrectomy for duodenal ulcer A more nearly normal reconstitution of the alimentary tract would result and the ulcer rate could be expected to be extremely low

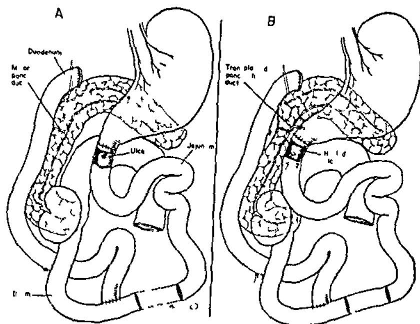



Fig 1

Seven animals recently operated upon have not yet had time to develop Mann Williamson ulcers

**A Pancreatic Duct Anastomosis to the Antrum** Ten animals developed *typical* Mann Williamson ulcers in the first portion of the jejunum within 1 to 8 wks Some of these animals explored at 4 wks had no ulcer only to develop an ulcer 3 to 4 wks later In only 1 case was no ulcer apparent even 11 wks after the Mann Williamson operation All animals lost weight The development of an ulcer was usually associated with inability to eat, more weight loss and general malaise

*Six of these* animals with ulcers then underwent anastomosis of the major pancreatic duct to the antrum as shown in Figure 1 The anastomosis was placed on the lesser curvature, 1 to 1½ in above the gastrojejunostomy stoma on the superior surface of the antrum An inner row of 5 0 silk was used to approximate the pancreatic duct to the antral mucosa Over this a *second row* of fine cotton was used to telescope the antral muscularis and serosa around the duct to the pancreatic capsule A polyethylene tube was used as a strut

In 5 of these animals complete healing of the Mann Williamson ulcer was noted at exploration and duodenotomy 4 wks later In each case the pancreatic duct anastomosis was checked for patency and function by probing through a separate gastrotomy incision

In 1 animal at exploration 4 wks after pancreatic duct anastomosis it was apparent that the original ulcer had enlarged 3 to 4 times Gastrotomy revealed the pancreatic duct anastomosis to be occluded by scar

**B Pancreatic Duct Anastomosis plus Histamine** Three animals whose Mann Williamson ulcers healed following pancreaticoantrostomy remained healthy for 4 7 and 8 wks respectively at which time they were given histamine in beeswax daily according to the method of Hay[5] Two of these animals developed perforated jejunal ulcers within 3 to 5 days of the onset of histamine injection The other animal survived 1 wk of *histamine* at

which time the injections were discontinued This dog is still alive and healthy 8 mo after pancreatic duct anastomosis One other dog was given histamine 1 mon following a Mann Williamson preparation No pancreatic duct anastomosis was done This animal died in 4 days of perforation and necrosis of the first portion of the jejunum just distal to the gastrojejunostomy

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# HEIDENHAIN POUCH SECRETORY RESPONSE TO GASTRO JEJUNOSTOMY AS AFFECTED BY POSITION ON THE STOMACH AND VARIATION IN STOMAL DIAMETER*

JOHN K STEVENSON JOHN E JESSEPH THOMAS W JONES AND HENRY N HARKINS

Previous investigators[1 2] reported no stimulatory effect on Heidenhain pouch secretion following low antral gastrojejunostomy utilizing a narrow stoma (less than 3 cm in diameter) At the same time, in our laboratory[3] utilizing a larger number of animals we found a definite stimulatory re sponse utilizing a wider stoma (4 cm in diameter)

Because clinical advice for performing a low gastrojejunostomy in patients is based on the former but disputed observation we thought it advisable to repeat the experiment in an attempt to settle the problem in a definitive manner

After careful study of all the factors involved the only common variant in the experimental preparations which might account for the different results was found to be the size of the gastrojejunostomy stoma With this in mind the experiment was repeated with controlled variation of this factor

## METHOD

A group of 7 mongrel dogs of both sexes and weighing between 8 to 15 kg was used Environmental conditions were carefully controlled as to kennel temperature time of feeding and time of collection of Heidenhain secre tions A Heidenhain pouch was constructed in each dog and standardized in the routine manner previously reported[3 4 5]

The results are quantitatively expressed in mEq HCl secreted for 24 hr and represent an average of 30 days collections After adequate Heidenhain

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pouch standardization a low antral antecolic isoperistaltic gastrojejunostomy was performed in all dogs. The jejunal anastomosis was made 10 cm distal to the ligament of Treitz and in the antrum 2 to 3 cm proximal to the pylorus. In each instance the procedure was the same except for the construction of the stoma which varied in size from 2 to 4 cm in diameter. Adequate time was allowed for the animal to recover from the operation before Heidenhain pouch secretions were again collected. After 30 consecutive 24 hr Heidenhain pouch collections had been made the animals were re-operated and the stoma size changed. All 2 cm diameter stomata were converted to 4 cm diameter and vice versa. The animals were again allowed to recover from the operative procedure before Heidenhain pouch collections were resumed. Collections were made for a period of 30 days in each animal after which the stoma was changed to its original diameter and another group of collections made. To terminate the experiment the gastrojejunostomy was taken down and a final period of Heidenhain pouch collections was made with normal bowel continuity restored.

In each instance stoma sizes were measured *in vivo* as accurately as possible with 2 cm and 4 cm diameter glass rods at the time of construction and at the time of conversion. Additional *in vivo* barium studies were made to determine the relative patency of the stomata and the main direction of flow.

## RESULTS

There was no mortality in this group of experiments. The animals tolerated the multiple operative procedures well as evidenced by maintenance of body weight, appetite and general well being.

When compared to the standardized Heidenhain pouch secretions there was an average increase of 48.8 per cent in the secretions collected during the periods that the animals had a gastrojejunostomy with a 2 cm stoma. However, when the animals had a 4 cm diameter gastrojejunostomy stoma there was a greater increase in HCl secretion by the Heidenhain pouch averaging 173.5 per cent. When the gastrojejunostomy was taken down and the Heidenhain pouch restandardized, there was a 40.0 per cent increase in HCl secretions over the pre-gastrojejunostomy level. (Fig. 1)

Barium studies done in each interval demonstrated in every instance the stoma to be patent. In general, when the animal had the 2 cm diameter gastrojejunostomy stoma there was a greater tendency for the barium to flow through the duodenum although some was seen to enter the jejunum directly. When the animal had a 4 cm diameter gastrojejunostomy stoma the flow of barium was directly into the jejunal loop with very little entering the duodenum. (Fig. 2) Evaluation of intragastric regurgitation or circus movement was not possible.

## SUMMARY

Under the conditions of this experimental study we found that there was only a moderate increase in Heidenhain pouch secretion following a low antral gastrojejunostomy if the stoma was constructed 2 cm in diameter. If, however, the gastrojejunostomy stoma was constructed 4 cm in diameter there was a marked such increase. The average increase in secretion of free HCl from the Heidenhain pouch when stimulated by 2 cm diameter gastro

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# HEIDENHAIN POUCH SECRETORY RESPONSE TO GASTROJEJUNOSTOMY AS AFFECTED BY POSITION ON THE STOMACH AND VARIATION IN STOMAL DIAMETER*

JOHN K. STEVENSON, JOHN L. JESSEPH, THOMAS W. JONES AND HENRY N. HARKINS

Previous investigators[1,2] reported no stimulatory effect on Heidenhain pouch secretion following low antral gastrojejunostomy utilizing a narrow stoma (less than 3 cm in diameter). At the same time in our laboratory[3] utilizing a larger number of animals, we found a definite stimulatory response utilizing a wider stoma (4 cm in diameter).

Because clinical advice for performing a low gastrojejunostomy in patients is based on the former but disputed observation, we thought it advisable to repeat the experiment in an attempt to settle the problem in a definitive manner.

After careful study of all the factors involved, the only common variant in the experimental preparations which might account for the different results was found to be the size of the gastrojejunostomy stoma. With this in mind the experiment was repeated with controlled variation of this factor.

METHOD

A group of 7 mongrel dogs of both sexes and weighing between 8 to 15 kg was used. Environmental conditions were carefully controlled as to kennel temperature, time of feeding, and time of collection of Heidenhain secretions. A Heidenhain pouch was constructed in each dog and standardized in the routine manner previously reported.[3,4,5]

The results are quantitatively expressed in mEq HCl secreted for 24 hr and represent an average of 30 days' collections. After adequate Heidenhain

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### CONCLUSION

Under the conditions of this experiment, stomal diameter in the case of a low gastrojejunostomy has a definite influence on the amount of Heidenhain pouch free HCl secretion.

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# RELATIVE SUSCEPTIBILITY OF THE GASTROINTESTINAL TRACT TO EXPERIMENTAL ACUTE PEPTIC ULCERATION*

LOUIS F. PLZAK, JR., WALTER FRIED AND EDWARD R. WOODWARD

When an anastomotic channel is created between the stomach and small intestine, especially in patients with duodenal ulcer, peptic ulceration of the exposed intestinal mucosa is a discouragingly frequent occurrence. In the genesis of these stomal ulcers the following 4 factors have been considered important: 1) the persisting secretion of acid peptic juice, 2) the sensitivity of the gut wall *per se* to acid pepsin digestion, 3) the neutralizing ability of bile, pancreatic juice and succus entericus, 4) the protective activity of mucus. This paper is concerned with an investigation designed to study the relative sensitivity of the various segments of the gastrointestinal tract to peptic digestion.

Following subtotal gastrectomy with a Billroth II reconstitution of gastrointestinal continuity, it has been commonly observed that as the stomach is anastomosed to progressively lower segments of the intestinal tract there is an increasing incidence of stomal ulcer. This observation has suggested to many workers that there is a gradient of susceptibility to ulceration. However, in a series of studies Merendino and co-workers[1, 2, 4] found that the neutralizing capacity of the intestinal contents at various levels could ac-

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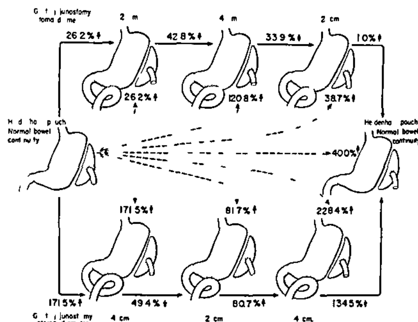



Fig 1 The sequence of operative procedures with resultant succeeding increases or decreases in free HCl secretion from Heidenhain pouches is shown In the upper portion of the chart the sequence is shown when the first anastomosis was 2 cm in diameter while below the sequence is demonstrated when the first anastomosis was 4 cm in diameter In all instances the final stage was a complete takedown of the anastomosis

The solid lines represent average per cent increase (↑) or decrease (↓) of free HCl secretion produced by an actual operative procedure The dotted lines indicate the percentage increase (↑) of each preparation as compared to the standardized control Heidenhain pouch with normal gastro intestinal continuity The increases as compared to the control for the 2 cm stomas were 26.2 38 7 and 81 7 per cent (average 48 8 per cent) The increases as compared to the control for the 4 cm stomas were 120 8 171.5 and 228 4 per cent (average 173 5 per cent) The overall effect of multiple procedures following takedown of the anastomosis in the 7 dogs was a 40 0 per cent increase

jejunostomy was 48 8 per cent For a 4 cm stoma the similar average was 173 5 per cent or over 3 times as much If the 40 0 per cent increase produced by multiple operations alone is considered this difference is even greater

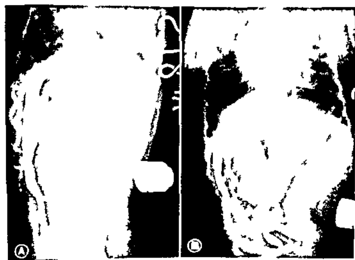


Fig 2 (A) Barium study of typical preparation with 2 cm gastrojejunostomy stoma Most of the barium goes out the pylorus only a portion through the stoma (B) Barium study of typical preparation with 4 cm gastrojejunostomy stoma All of the barium goes through the stoma none through the pylorus

*Table 1 Histologically Determined Extent of Penetration of Ulcers in Various Regions of the Digestive Tract*

| | Region of Gastrointestinal Tract | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Experiment | High Esophagus | Low Esophagus | Body Stomach | Antrum Stomach | Duodenum | Jejunum | Ileum | Colon |
| A | | 4 | 1 | | 1 | | 1 | |
| B | | | 1 | | | 1 | [illegible] | 5 |
| C | | 4 | 3 | 3 | | 1 | 1 | [illegible] |
| D | 4 | 4 | 3 | 3 | 4 | 1 | 1 | |
| E | 4 | 4 | 1 | 2 | 3 | 3 | 3 | 5 |
| F | | | | | 2 | 2 | 2 | 4 |
| G | | | | | 5 | 5 | 5 | 5 |
| H | | | | | 5 | 5 | 5 | 5 |
| I | | | | | 3 | 3 | | |

| Number | Depth of Erosion or Ulceration |
|---|---|
| 1 | In the epithelium and lamina propria |
| 2 | Up to the muscularis mucosa |
| 3 | In the muscularis mucosa |
| 4 | Up to the submucosa or in it superficially |
| 5 | In the submucosa |

compared not only grossly but also by microscopic study of serial sections of the ulcers with determination of the layers of gut penetrated at the point of maximal damage

RESULTS

The *terminal results are summarized in* Table 1

**Esophagus** The sequence of events and the terminal condition of the esophagus were the same in both areas selected for study Ischemia was noted within a minute after the stream was started and the mucosa was penetrated within 5 min The underlying layers thus exposed soon presented a glossy translucent appearance which did not change as the experiment *progressed* Even after two hr there was no further penetration of these layers by acid pepsin solution

**Stomach** Of the 8 sites tested the 2 sites selected in the stomach were the most resistant to ulceration The damage to the stomach mucosa appeared much later than did injury to the other sections of the gastrointestinal tract tested The antral and fundic mucosa seemed to be about equally resistant to the acid pepsin solution

***Intestine*** The 3 areas of the small intestine investigated seemed equally susceptible to erosion Within a fraction of a minute after *beginning* the streams the areas became ischemic and petechial hemorrhages appeared The mucosal surface was rapidly eroded and after 15 to 40 min (*depending* upon the experimental conditions) necrosis extended to the muscularis mucosae This layer is relatively resistant to destruction and was not always penetrated

count for this apparent gradient of susceptibility and concluded that the inherent mucosal sensitivity is relatively unimportant in peptic ulceration.

Le Veen[4] reported a method of producing acute damage to the gut with a constant fine stream of acid pepsin solution which washed away all neutralizing and neutralized substances and thus continuously exposed the selected area to fresh acid pepsin solution. By utilizing a modification of Le Veen's technique, we have further investigated the question of the importance of tissue susceptibility *per se* in peptic ulceration of the gastrointestinal tract.

## METHOD

Adult mongrel dogs under nembutal anesthesia were utilized as the experimental animal. The dogs were intubated and respiration was maintained by positive pressure anesthesia.

Artificial gastric juice was prepared at pH 1.2 and concentration of *4 to 5* mg. pepsin per cc. The acid pepsin solution was warmed in a water bath at 37 C. and maintained at this temperature throughout the experiment. Rubber tubing leading from the pepsin solution was passed through an electrically driven pump. Distal to this pump the stream was divided by a series of interposed glass Y tubes into multiple jets issuing from No. 22 gauge needles. The size of stream, rate of flow, and the pressure of the jet of fluid escaping from each needle were uniform for each experiment; the pressure used varied from 34 to 48 cm. of water in the different experiments.

Eight areas of the gastrointestinal tract were selected for simultaneous exposure to the stream of peptic juice. They were:

1. Esophagus, 7 cm. distal to the cricoid cartilage
2. Esophagus, 1–2 cm. proximal to the cardiac sphincter
3. Fundus of the stomach, midposterior wall
4. Pyloric antrum of the stomach, posterior wall
5. Duodenum, 10 cm. distal to the pylorus
6. Jejunum, 10 cm. distal to the ligament of Treitz
7. Ileum, 10 cm. proximal to the ileocecal valve
8. Colon, 10 cm. distal to the ileocecal valve

Ten to 15 cm. segments of gut containing the areas to be perfused were carefully ligated to preserve fully the blood supply and were slit open along the antimesenteric border. The mucosal surface was everted and the mesenteric border of the gut was exteriorized. The exposed tissue was positioned so as to maintain a constant minimal tension on it and to provide optimal drainage of juice from the proposed ulcer site. Drainage was provided either by gravity or by strategically placed suction equipment. The remainder of the abdominal contents was protected from the peptic solution by saline sponges. The needles through which the peptic solution passed were held in place 3 cm. from the mucosa and at 45° angles to the exposed surface by burette clamps. The jets were all started at once, and gross observations of the exposed sites were recorded as the experiment progressed. The average duration of the 9 experiments was approximately 2 hr.; time varied from 45 min. to 2½ hr. Adequate vascular supply to the immediate area of the lesion was ascertained at the conclusion of the experiments by noting considerable bleeding as the ulcer sites were excised for histological study. The dogs were then sacrificed. The relative degrees of ulceration in the various areas were

secretions contained in the gut On the basis of our experiments we concluded that

1 The mucosa of the esophagus (both upper and lower) is least resistant to peptic ulceration but the damage does not penetrate further into the tissue even when exposure to the jet of peptic solution is greatly prolonged

2 The antrum and fundus of the stomach are about equally resistant to ulceration these are the most resistant parts of the gastrointestinal tract

3 Punched-out ulcers form with essentially equal facility in the duodenum jejunum and ileum in the absence of neutralizing secretions These tissues are intermediate between the esophagus and stomach in their susceptibility to ulceration

4 Although the epithelium and lamina propria of the small and large bowel are equally susceptible to erosion the muscularis mucosa in the colon is more readily penetrated and hence the colon is more susceptible to true ulceration than the rest of the intestine

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## THE GENESIS OF INTESTINAL ATRESIA*

C N Barnard

Since the time that Osiander recorded the first case of congenital occlusion of the intestine in 1797 many views as to the causation of this anomaly have been expressed Most of these were based on isolated morbid anatomical findings or indirect experimental evidence and can be briefly summarized under 3 main headings 1) *Developmental defects* Under this heading are grouped the theories of Meckel Bland Sutton and Tandler who all attempted to explain the anomaly on some failure of correct development of the fetal gut 2) *Fetal accidents* Intra abdominal catastrophes such as intussusceptions volvuli kinks etc were blamed by some for the atresia 3) *Fetal disease* Several investigators incriminated fetal disease such as peri

* From the Department of Surgery University of Minnesota Medical School Minneapolis 14 Minn Supported by Austen S and Anne F Cargill Fund for Surgical Research Jay and Rose Phillips Fund Richard C Lilly Fund

within the duration of the experiment The observations in the duodenum jejunum and ileum were consistently identical data from the colon indicated that it was somewhat more susceptible to ulcer formation than small intestine The mucosa was eroded down to the muscularis mucosa at the same rate as in the small bowel damage from this point on progressed more rapidly in the large intestine

**Control** As a control 2 sites 5 cm apart in the jejunum were selected for subjection to a stream of heated (37°C) HCl solution at pH 1 2 and the acid pepsin solution used in the experiments The conditions at each site were identical No erosion occurred at the site that received only the acid whereas the pepsin solution in the same time interval produced the typical acute ulceration noted above

The correlation between the gross observations during the experiment and the histologic findings was remarkably close

## DISCUSSION

We desired to use a jet of fluid in this experiment so as to deliver a peptic solution of known activity to the selected area constantly and to wash away continually any mucus secreted by the gut The ulcers produced did not result merely from the pressure of the system since a comparable acid control solution without pepsin was impotent when compared to the acid pepsin solution under the same conditions The results indicate the degree of actual innate tissue resistance to ulceration in the various segments of the alimentary tract insofar as the protective effects of gut contents and mucosal secretions have been eliminated by our experimental method

The finding that the esophageal mucosa is most susceptible to peptic digestion was expected on the basis of numerous other observations and investigations it was surprising however to note that after erosion of the mucosa no further damage seemed to occur in the time intervals studied The great degree of resistance on the part of the stomach was to be expected Difficulties in maintaining equivalent amounts of tension during the experiment in the fundic and antral areas prohibited a precise conclusion concerning the relative susceptibilities in these regions It can be said that there are no considerable differences in resistance in these parts of the stomach The essentially identical degree of damage in the duodenum jejunum and ileum as the experiments progressed indicated that these areas of the small bowel are apparently equally susceptible to acute peptic ulceration The colon usually ulcerated more rapidly than the small bowel this seemed to be due to greater ease of penetration through the muscularis mucosae in the colon since the epithelium and lamina propria were eroded at the same rate in both the small and large gut The facility of penetration of the muscularis mucosa in the colon may be due to the thinness of this layer as compared with that in the small intestine

## SUMMARY AND CONCLUSIONS

We have presented evidence of the relative susceptibility of the gastrointestinal tract in the dog to experimental acute peptic ulceration by subjecting selected areas of it to a stream of pepsin solution at pH 1 2 By our technique we have tried to examine the innate sensitivity of the tissue to ulceration at various levels by excluding the effects of neutralizing and protective



Fig 2 Autopsy findings in puppy born 10 days after intrauterine operation Proximal bowel distended ending in bulbous tip area of atresia and collapsed distal bowel



Fig 3 Photomicrograph showing the fusion of distal and proximal blind ends with the formation of a septum

tion of the infarcted segment with at the most a complicating meconium peritonitis

The question may be asked But how do we know that the vascular interference precedes the atresia and not atresia the vascular occlusion?

## METHOD

Operations were done upon dogs in utero In some experiments the mesenteric vessels which supply a short segment of small bowel were ligated in other experiments the blood supply to a segment of ileum was interrupted by creating a strangulating obstruction The mother dog was then allowed to go to term and the puppies operated upon were sacrificed within 24 hr after birth

On post mortem examination of these puppies it was found that the infarcted or strangulated segment was in various stages of disintegration depending on how soon the puppy was born after the interference to the blood supply In puppies born from 7 to 10 days after surgery in which the mesenteric vessels were ligated an anomaly of the gut identical with that found in infants with ileal atresia resulted the bowel proximal to the infarcted segment was found to be distended ending in a bulbous tip The bowel distal to the infarcted area was normal but completely collapsed There was a V-shaped defect in the mesentery and the infarcted bowel was entirely represented by a fibrous strand (See Fig 2) In puppies born from 7 to 10 days after a segment of bowel was strangulated the same defect resulted however in some of these puppies on account of the strangulation the proximal and

tonitis or intestinal ulceration When all these theories are studied in more detail it becomes certain that one mechanism cannot explain all cases of congenital intestinal atresia

Louw[1] reviewed 79 consecutive cases of atresia and severe stenosis in the newborn at the Hospital for Sick Children London and noted that certain features of the anomaly suggested that some atresias may be due to interference with the blood supply to a portion of fetal gut These features include the following 1 ) Anomalous vascular supply to the atretic portion by a V shaped defect in the mesentery 2 ) Early necrosis of the proximal blind end before the tension in this area has risen to any extent This occurred in 20 per cent of the cases and in half of these cases the infant was less than 48 hr old 3 ) Postoperative atony and ileus in apparently healthy blind ends anastomosed to the distal bowel Similar observations have been reported by other workers and the following cases studied at Groote Schuur Hospital Cape Town under Prof J H Louw* further strengthened our belief in the vascular hypothesis (a) An infant born with aplasia of the descending and pelvic colons in whom there was complete absence of the inferior mesenteric artery (b) An infant operated on for ileal atresia whose resected specimen clearly showed the remnant of an intussusception in the distal segment (c) A newborn infant with intestinal obstruction whose ileum clearly showed the presence of a volvulus which was presumably responsible for the atresia (Fig 1) (d) An infant with intestinal atresia in whom at operation a congenital band had caused infarction of the bowel and was probably responsible for the atresia

Although strangulation causes infarction of the bowel followed by gangrene perforation and peritonitis in postnatal life the train of events are different in the sterile fetal bowel Here strangulation results in the absorp




Fig 1 Segment of ileum resected at operation showing atresia at the ileum and the presence of a volvulus

*Prof J H Louw Head of the Department of Surgery University of Cape Town Cape Town South Africa

# *The Liver and Pancreas*

## NITROGEN BALANCE IN ANIMALS WITH BILIARY FISTULA*

LOREN J. HUMPHREY

Persistent loss of bile is associated in most patients with a marked weight loss. The increase in fecal lipid excretion with a biliary fistula is well known as an important factor in the general debility of these patients. Protein metabolism has received less attention in investigations concerned with patients and animals with biliary fistula. In this paper nitrogen balance, plasma volume and circulating protein studies are reported in dogs with a biliary fistula.

### METHOD

Mongrel dogs of both sexes varying in weight from 7 to 13 kg. were used in this study. All dogs received 60 mg. of morphine sulphate preoperatively and 22 mg./kg. of sodium pentothal during surgery.

An internal biliary fistula was chosen for these studies in an attempt to avoid the cholangitis associated with external methods of bile diversion. The procedure was that of cholecystonephrostomy as described by Kapsinow, Engle and Harvey.[1] In this operation the gallbladder is anastamosed to the right renal pelvis through a transcortical incision in the kidney after ligation and section of the common bile duct.

All dogs were placed on a special diet containing 65 per cent fat, 20 per cent protein and 15 per cent carbohydrate and given 3.4 gm. of protein/kg./day and 75 calories/kg./day. One control period (i.e. 1 wk.) was obtained in the first 4 dogs and 3 control periods (i.e. 3 wks.) were obtained in the last 3 dogs. During these control periods plasma volume, blood protein and nitrogen balance determinations were made.

Beginning with the third postoperative week successive 7 day periods were run with each dog being placed on the special diet.

The Evans Blue dye method was used in determining weekly plasma volumes. Total serum protein, serum albumin and serum globulin concentrations were determined weekly using the biuret method. Dietary and urinary nitrogen were determined by the Micro Kjeldahl method. Since Heersma and Annegers[2] state that fecal nitrogen varies only with the fiber content of the diet in dogs with biliary fistula, we determined only dietary and urinary nitrogen and used F as a correction factor for fecal nitrogen.

### RESULTS

A total of 28 dogs were operated upon in this study. The operative mortality was 14.3 per cent. Eight dogs were used as a preliminary study to de-

*From the Department of Surgery, University of Illinois College of Medicine, Chicago, Ill. Aided by a grant from the Graduate School, University of Illinois. With the technical assistance of Mr. Everett Hoppe.

distal blind ends were pulled so close together that they fused and a septum resulted (Fig 3)

These successful experiments together with the other observations constitute sufficient proof that at least a proportion of intestinal atresia are due to a vascular accident causing infarction of a segment of a fetal bowel and absorption of the affected segment resulting in atresia It also shows one mechanism by which a septum can result

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termine the most palatable diet and the minimum requirements for a positive nitrogen balance

Of the remaining 20 dogs 7 dogs fulfilled the criteria for inclusion in this series The majority were excluded because of failure to ingest dietary nitrogen in amounts equivalent to that of their control period

All dogs lost weight rapidly and appeared quite cachectic The average survival time of these 7 dogs was 8 wks and the average weight for these 7 dogs at 6 wks postoperatively was 28 4 per cent below the preoperative average

Data on plasma volume determinations shows a progressive decrease in total plasma volume averages postoperatively with an average decrease of 26 5 per cent 6 wks postoperatively below the average of control values However serum protein levels at 6 wks postoperatively increased 17 4 per cent above control levels

At 6 wks postoperatively the serum albumin concentration was 31 7 per cent above the control level and the serum globulin concentration average was 12 5 per cent below the control level

Nitrogen balance determinations in all 7 dogs showed a more negative nitrogen balance after operation than in the control periods The average preoperative urine nitrogen excretion on these dogs was 35 36 gm for each 7 day period These same dogs postoperatively excreted an average of 45 63 gm of nitrogen in the urine for each 7 day period This means that postoperatively on an average 10 27 gm of nitrogen/wk or 64 19 gm of protein/wk were lost in excess of control values Bi weekly nitrogen balance averages on all dogs and nitrogen balance averages for individual dogs are given in Figures 1 and 2

## DISCUSSION

It is well known that the lack of bile in the intestinal tract causes a marked weight loss This has been reported by Cole [3] Bissell [4] Colwell [5] and

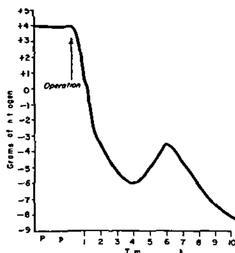



Fig 1 Bi weekly nitrogen balance averages for 7 dogs showing preoperative control level and levels for 10 postoperative wks following the production of an internal biliary fistula

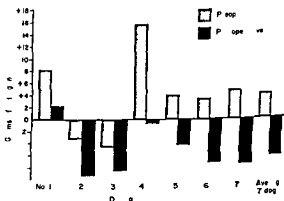



Fig 2 Nitrogen balance averages for individual dog showing preoperative control levels and the average levels of 5 postoperative wks following the production of an internal biliary fistula

others In this study there was severe weight loss leading to death from cachexia on the average within 8 wks

Paralleling the marked weight loss in this study is a marked decrease in total plasma volume It is also seen from this study that total serum protein concentrations rise progressively when bile is absent from the intestinal tract This progressive rise is probably due to concentration of protein as plasma volume diminishes

It is seen that in these dogs with biliary fistula there is a progressive increase in serum albumin concentration and a progressive decrease in serum globulin concentration beginning at the fourth week postoperatively Since the half life of alpha globulin is much shorter than that for albumin it is possible that this is a result of starvation

The primary purpose of this study was to determine the effect of diversion of bile from the intestinal tract on nitrogen balance It is seen that the difference between control nitrogen balance averages and postoperative nitrogen balance averages is 10 27 gm for a 7 day period In terms of protein this means each dog lost 64 19 gm more of protein each week postoperatively than was ingested However from this study we cannot say whether the more negative nitrogen balance postoperatively resulted from a decrease in fat absorption and hence a loss of protein sparing action of fat, or whether the loss of bile from the hepatic circulation alters the metabolism of liver cells or assimilation of protein

## SUMMARY

An experiment has been conducted to determine the effect of the diversion of bile from the intestinal tract on nitrogen balance A cholecystonephrostomy was performed on 7 dogs which had been studied preoperatively for control periods

All dogs progressively lost weight and the average length of survival postoperatively was 8 wks The decrease in total plasma volume paralleled this weight loss while total serum protein concentration increased slightly in all dogs postoperatively At 4 wks postoperatively serum albumin concentration increased progressively and serum globulin concentration decreased progressively

A more negative nitrogen balance was found to exist postoperatively in all the dogs with a biliary fistula in this series in these dogs the difference between the control nitrogen balance average and the postoperative nitrogen balance average was 10 27 gm /wk

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# THE ROLE OF THE SHWARTZMAN REACTION IN THE PATHOGENESIS OF INFLAMMATORY LESIONS OF THE BILIARY TRACT*

Stuart Roberts and Richard Webb, Jr

In studying the dermal Shwartzman reaction it appeared that this phenomenon of local tissue reactivity consisting of hemorrhagic necrosis with healing by fibrosis may be implicated in the occasional progressive inflammatory destruction of the common duct which has been described as *obliterative cholangitis*. Most surgeons[1] agree that the vast majority of strictures of the common duct result from trauma inflicted during operation, yet in a small percentage of cases infection itself appears to be the primary factor.[2] In some instances the infection behaves in an unusual fashion, progressing to the destruction of the duct converting it into a fibrous cord.[1]

The Shwartzman reaction has been elicited in many tissues and organs including skin, stomach, appendix, bowel and pancreas,[3, 4] but it has not been applied to the pathogenesis of strictures of the common duct nor to severe inflammatory reactions occasionally seen in the biliary tract.

A brief description of the phenomenon and definition of terms are essential in order to fully understand the experiments. The Shwartzman reaction is a 2 stage reaction (Table 1) in which the first event is sensitization of the desired tissue brought about by the local injection of culture filtrates or *toxins of certain gram negative micro organisms; the second event is initiated* by the intravenous injection of the same or other toxin (diluted) 24 hr later and consists of hemorrhagic necrosis of the sensitized tissue. The first injection is referred to as the sensitizing injection. The second injection always given intravenously 24 hr later is called the challenging injection.

## METHOD

**Toxin** Meningococcal toxin was prepared from agar washings of cultures 44 B strain of meningococcus obtained through the kindness of Dr Gregory Shwartzman, Mount Sinai Hospital, New York. The toxin was prepared according to the method of Shwartzman.[3] Each batch of toxin was tested for potency immediately prior to use by an intradermal injection (25 cc) in

*Table 1 Elicitation of the Shwartzman Reaction†*

| | |
|---|---|
| STAGE ONE (SENSITIZATION) | |
| Route of Injection | Application of Methods |
| (1) Into parenchyma of tissue, i.e. | Into wall of common duct or gallbladder |
| (2) Application to surface of tissue, i.e. | Instillation into lumen of common duct |
| (3) Into vascular supply of organ, i.e. | Into cystic artery |
| INCUBATION PERIOD 24 hr | |
| STAGE TWO (CHALLENGING) | |
| Intravenous Injection, i.e. | Into jugular vein |

†Modified from Rostenberg

*From the Department of Surgery, University of Illinois College of Medicine, Chicago, Illinois. With the technical assistance of Everett Hoppe and Ruth McCrath. Aided by a grant from the Graduate School, University of Illinois.

rabbits followed by an intravenous injection (2 cc. of 1:10 dilution) of the same toxin 24 hr. later. Potent toxin uniformly produced an area of dermal hemorrhagic necrosis 1 × 3 cm. in diameter. The toxin was stored at 4°C. and each batch of toxin was used within 3 wks.

**Animals.** Except for testing of potency of toxin in albino rabbits, adult male and female goats of domestic breeds weighing 20 to 62 kg. were used throughout the experiments. The goats were lightly anesthetized with intravenous pentobarbital using an endotracheal tube. Dogs are not susceptible to the Shwartzman reaction.

**Histologic Methods.** As soon as the animals died or were sacrificed, tissues were fixed in 10 per cent formaldehyde solution. Sections were stained by the hematoxylin and eosin method.

**Defunctionalization of the Common Duct.** The distal portion (3 to 4 cm.) of common duct was defunctionalized by placing a ligature around the common duct just distal to the point of entrance of the pancreatic duct into the common duct. Decompression of bile and pancreatic secretions was accomplished by cholecystduodenostomy (Group 2).

## RESULTS

The Shwartzman reaction was elicited in the common duct or gallbladder in 7 of the 16 goats utilized in the 3 groups.

**Group 1. Injection Into Wall of Common Duct or Gallbladder.** In this group the toxin was injected (sensitization) into the wall of the common duct or gallbladder of 11 goats followed by an intravenous (challenging) injection of toxin 24 hr. later. There were positive results in 6 animals evidenced grossly by bluish discoloration 1 to 3 cm. in diameter at the injection site of toxin, and microscopically by hemorrhage, thrombosis of venules and necrosis of cells. As a control against the factor of trauma from injection, a similar amount (0.25 cc.) of physiologic saline was injected into another area of the common duct or gallbladder with no resulting evidence of reaction.

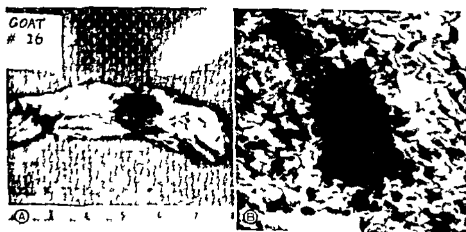



Fig. 1. A. Gross specimen of a common duct from a goat (No. 16) in Group 1. The pointer is directed to the injection site of toxin in which a sharply circumscribed reaction is seen. B. Photomicrograph (700×) of injection site of toxin into wall of gallbladder from a goat (No. 15) in Group 1. This section shows thrombosis of a small vessel and some perivascular cuffing of polymorphonuclear leukocytes.

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*From the Department of Surgery University of Illinois College of Medicine Chicago Illinois With the technical assistance of Everett Hoppe and Ruth McGrath Aided by a grant from the Graduate School University of Illinois

ney[4] demonstrated that toxin readily diffuses through an apparently intact pancreatic ductal system in rabbits and goats The accidental trauma to the common duct at the time of cholecystectomy might sensitize the duct wall if bacteria were present at that time Once the common duct is sensitized the circulation of products of gram negative micro organisms in the blood stream within the proper time interval would elicit the Shwartzman reaction

SUMMARY

From the data reported herein it would appear that the Shwartzman reaction can be elicited in the biliary tract and may occasionally be a factor in the pathogenesis of obliterative cholangitis The sensitization process might appear more likely to be produced with the aid of severe trauma (accidental section) to the common duct but possibly could be secondary to the very minor trauma incident to the average operation This process could explain the unusual progressive reaction in certain cases Conceivably, it could also be a factor in the development of cholecystitis and hepatitis

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## COMPARATIVE FAT AND FATTY ACID STUDIES IN INTESTINAL MALABSORPTION STATES*

JAMES R MALM KEITH REEMTSMA AND HAROLD G BARKER

The evaluation of malabsorptive states in medical and surgical patients has long presented difficult diagnostic problems In recent years following the original method as described by Stanley and Thannhauser [1] a number of investigators have used radioactive iodinated fat to study absorptive patterns in normal and abnormal states The test as described demonstrated fat malabsorption but gave no clue as to the defect involved

* From the Department of Surgery Columbia University College of Physicians and Surgeons and Surgical Service Presbyterian Hospital New York City Supported in part by U S Public Health Grant RC 2926 (C 4)

**Group 2 Instillation Into Lumen of Common Duct** Three goats are represented in this group in which the toxin was instilled (sensitization) through a polyethylene tube introduced into that portion of the distal common duct which was defunctionalized Twenty four hours later an intravenous (challenging) injection of toxin was given, resulting in a positive reaction in 1 animal evidenced grossly by numerous petechial hemorrhages with an area of confluence 0 5 cm in diameter Microscopically there was hemorrhage, thrombosis of venules and necrosis of cells

**Group 3 Injection Into Cystic Artery** In this group toxin was injected (sensitization) into the cystic artery of three goats followed by an intravenous (challenging) injection of toxin 24 hr later There was an equivocal reaction in 1 animal evidenced grossly by a darker blue black color of the entire gallbladder There were also several areas of bluish discoloration of adjacent liver and examination of the cystic artery revealed several small branches to the involved liver Microscopically the wall of the gallbladder was thicker, because of edema No areas of hemorrhagic necrosis were seen however, thrombosis of several small venules was noted

## DISCUSSION

A considerable number of goats (9 of 15) died within 2 to 24 hr following the intravenous challenging injection This lethal effect of agar washings filtrates was noted in rabbits by Shwartzman[3] He also observed that the concentration of lethal factors somewhat parallels the concentration of toxin in filtrates derived from micro organisms capable of producing toxin in high concentration (both of which are high from meningococcus)[5] Since over one half the animals died within 24 hr the conclusion might be drawn that an adequate amount of toxin was given, the reason for negative results was apparently due to the factor termed by Shwartzman[5] as individual susceptibility, and not to an insufficient amount of toxin

In view of the frequent presence of E coli in the bile of patients with stricture of the common duct toxin was prepared from an E coli obtained *from the bile of patients undergoing choledochoplasty for stricture of the* common duct However, the toxin derived from these strains proved to be less potent than that obtained from the meningococcus It has been amply demonstrated that toxin derived from other gram negative micro organisms including some strains of E coli have almost equal ability to elicit the Shwartzman reaction[3, 4]

The clinical implications of these experiments are related to the possibility that the destructive process in the wall of the common duct may, in fact be due to sensitization by toxins from bacteria present at the time of operation Shwartzman[5] noted that the reaction following the challenging injection is not localized to the injection site of toxin The spreading of the area of sensitization is believed to be via the lymphatics of which there is a rich network in the submucosa of the common duct[6, 7]

Since it has been shown that the disappearance of the toxin from the blood stream is correlated to its appearance in liver extracts (also extracts of spleen)[3] the bacterial toxins under certain circumstances may subsequently sensitize the common duct either (1) by way of the lymphatic network from the liver or (2) in the bile excreted by the liver Thal and Brack

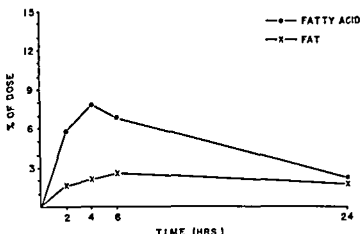



Fig. 2 Percentage of ingested I[131] dose in circulation at various time intervals in patients with pancreatic insufficiency

and the serum concentrations are about one half as high The difference is statistically significant and establishes a basis for evaluating the abnormal The reasons for this difference are not clear and it may be both a function of absorption and disposal from the blood stream Recent experimental work suggests that oleic acid forms a calcium-oleate phosphate complex in the intestinal lumen that inhibits up to 30 per cent of absorption[4] Our data confirm the relatively high fecal loss of $I^{131}$ tagged oleic acid in normals (14 5 per cent to 6 2 per cent of the ingested dose)

A group of patients with pancreatic insufficiency have been studied utilizing the comparative absorption tests Figure 2 shows the mean serum values obtained in 6 proven cases It is evident that while there is a marked decrease in fat absorption as compared to the normal curve oleic acid is absorbed in a normal fashion This pattern is diagnostic of pancreatic insufficiency and has not been encountered by us in any other disease state in about 200 tests thus far performed

Figure 3 shows the mean serum values obtained in a group of 14 patients with documented primary intestinal malabsorption This group included cases of extensive small bowel resection sprue Whipple s disease regional enteritis and fibrocystic disease The pattern obtained shows a marked impairment of fat and fatty acid absorption A similar pattern has been obtained in the presence of pyloric obstruction and in patients with very rapid intestinal transit times In the absence of these factors the pattern is

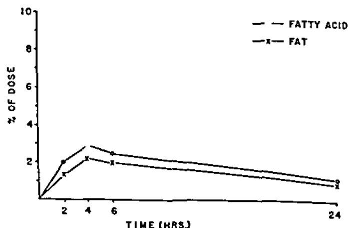



Fig 3 Percentage of ingested I[131] dose in circulation at various time intervals in patients with primary intestinal malabsorption

The present test separates the digestive phase of fat utilization from the absorptive phase Prior to entering the blood stream long chain fats require the action of pancreatic enzymes to hydrolyse the fat molecules, at least in part into constituent fatty acids The products of this hydrolysis are then transported by a function of the intestinal wall to the lymphatics and capillaries *In the absence of pancreatic enzymes little or no fat absorption can* be anticipated but absorption of free fatty acids which remains a function of the intestinal wall independent of pancreatic enzyme should proceed normally In the presence of primary intestinal dysfunction impaired absorption would be predicted for both fat and its free fatty acid

## METHOD

We have administered $I^{131}$ tagged olive oil and its principal fatty acid oleic acid prepared in our laboratory by a method previously described The resultant material contains less than 50 microcuries of $I^{131}$ per test dose (9 to 15 cc) The tests are performed at least 48 hr apart and patients are prepared with Lugol s solution for 48 hr prior to testing to suppress uptake of iodine by the thyroid gland Test meals have been given to fasting patients and a modified breakfast allowed 1 hr after the test has begun Blood samples were drawn at 2 4 6, 8 12 and 24 hr Urine collections were made over a 48 hr period and in some cases 72 hr stool collections were made Serum was separated for analysis and all samples were counted in a well type scintillation counter results being expressed in percentage of the total dose given Serum samples were expressed as a percentage of the total dose which is in the entire blood volume at the time of sampling based on an assumed serum volume of 4 5 per cent body weight Urine and stool data were each expressed in cumulative figures calculated into per cent of dose administered

## RESULTS AND DISCUSSION

The results obtained in normal patients following ingestion of $I^{131}$ tagged olive oil and oleic acid are shown in Figure 1 *Our results in normal* patients following ingestion of tagged olive oil agree with results obtained by others utilizing commercially prepared $I^{131}$ tagged triolein[3] The serum curve obtained following $I^{131}$ tagged oleic acid is of particular interest as the radioactivity in the serum reaches a peak 2 hr later than that following olive oil

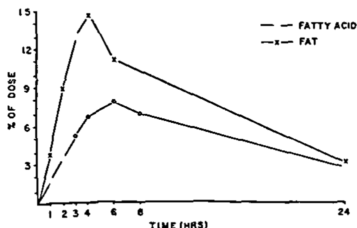



Fig 1 Percentage of ingested $I^{131}$ dose in circulation at various time intervals in normal individuals Zero time is time of ingestion

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# THIRY FISTULA STUDIES IN STRANGULATION OBSTRUCTION*

George J Wernette and Isidore Cohn Jr

The advantages of a Thiry fistula preparation for the study of specialized phases of strangulation obstruction have been demonstrated and it has been shown that many of the variables usually encountered in strangulation obstruction can be eliminated[1,2]

These experiments were designed to study the role of one of the factors in strangulation obstruction as evaluated under the controlled conditions of the Thiry fistula preparation The role of specific bacteria was studied in a strangulated segment of bowel without interfering with normal gastro-intestinal function causing obstruction or distention or producing the other factors which usually accompany strangulation obstruction It was hoped that further evaluation of the role of *Cl welchii* in strangulation obstruction could be obtained from these studies

## METHOD

Dogs were operated upon under aseptic conditions and a 50 to 75 cm Thiry fistula established The omentum was removed as completely as possible to prevent it from forming too many adhesions or acting as a source of vascularization following the second operation The animals were allowed 2 to 16 months to return to normal This provided time for the fistula to expel all of its contents except those secreted by the fistula itself Since there was no continuing source of re introduction of bacteria there was also time for the bacterial content of the fistula to be reduced

At the second operation a 30 cm central segment of the fistula was deprived of its venous supply by dividing and ligating all the veins to this segment and by dividing and ligating the arteries and veins parallel to the segment at both of its ends This provided the strangulated isolated open loop of small bowel in which studies could be conducted A small plastic tube was threaded into the fistula to permit instillation of bacterial cultures deep into the lumen of the fistula

Rubber tubes were placed in the peritoneal cavity for postoperative aspiration of peritoneal fluid At frequent intervals specific gravity and white cell counts were determined for peritoneal fluid and blood and the peritoneal fluid was cultured The animals were maintained on intravenous

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*Table 1 Cumulative Urinary Recoveries as Per Cent of Dose*

| SUBSTANCE GIVEN | NORMALS | | PANCREATICS | | INTESTINALS | |
|---|---|---|---|---|---|---|
| | 24 HR | 48 HR | 24 HR | 48 HR | 24 HR | 48 HR |
| Fat | 70 | 75 | 20 | 40 | 26 | 37 |
| Fatty Acid | 54 | 72 | 38 | | 32 | 43 |

diagnostic of malabsorption of the primary intestinal type It is apparent that pancreatic function can not be evaluated in the presence of intestinal malabsorption by this test

The study of serum values has proved the most informative means of following the rate of absorption but some valuable quantitative data can be obtained from an analysis of the $I^{131}$ recovered in the urine Table I shows the comparative cumulative urinary recovery of $I^{131}$ following ingestion of tagged oil and oleic acid It is apparent that the total urinary excretion of $I^{131}$ is low when malabsorption is present There seemed to be a wide variation in individual cases which may represent a difference in the rate of absorption not reflected in the serum values The urine excretion does not appear to be as sensitive a guide as the serum values in most cases and should not be substituted for blood studies The recovery of $I^{131}$ in the stool correlated well with the anticipated amount of undigested fat based on total dose minus cumulative urine excretion Stool studies have not been a routine part of the test but were done only to complete the balance studies in selected cases

A further use for these tests has been in the evaluation of effects of certain medications on fat absorption such as cortisone and pancreatin The success of pancreatic drainage procedures can be evaluated by repeated studies The problem of post gastrectomy malabsorption syndrome is being studied at this time In addition the tests offer a valuable method for further investigation into the mechanism of fat absorption and utilization

## SUMMARY

A comparative absorption test utilizing $I^{131}$ tagged olive oil and oleic acid has been outlined The absorptive patterns in normal patients is reported and compared to that obtained in pancreatic insufficiency and primary intestinal malabsorption A low fat and normal fatty acid absorption is consistently found in pancreatic insufficiency while both absorptive curves are low in primary intestinal malabsorption Cumulative urinary recovery of $I^{131}$ is a quantitative guide to the degree of malabsorption but has considerable individual variation Some additional uses of the test are discussed

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In general the white cell counts of the peritoneal fluid and blood showed a gradual rise throughout the important first 18 hr This is in line with previous observations that the count will continue to rise in an animal that is successfully combatting the condition in contrast to its drop in those animals that are succumbing to the procedure The animal that died most rapidly had a distinct drop in the blood white cell count but the peritoneal fluid count continued to rise throughout the experiment

Bloody drainage from the fistula occurred at some time or other in each of these experiments in contrast to its usual absence in the previous Thiry fistula experiments[2] It was first observed from 2 to 11 hr after strangulation and had usually stopped by 18 hr

The amount of *Cl welchii* culture injected was far less than that which had been shown to be without effect in the presence of a normal gastro intestinal mucosa but well above the lethal quantity when injected into the peritoneal cavity in the presence of some suitable culture medium The rapid expulsion of much of the injected culture and the integrity of the bowel wall acted together to prevent an over dosage of culture in the peritoneal cavity at any one time The presence of *Cl welchii* in the early peritoneal fluid in some experiments was attributed to the technique of injecting the culture into the fistula immediately after strangulation and before the peritoneal cavity was closed In subsequent experiments when cultures were not injected until after the abdomen was closed the early cultures were not positive for *Cl welchii*

Seven dogs were subjected to these experiments Four dogs survived to be re explored at intervals of 3 to 4 wks at which time the strangulated portion of the fistula was grossly normal Three dogs died at 34½ 138 and 228 hr (9½ days)

Autopsy on the 2 animals that died at 34½ and 138 hr revealed hemorrhage gangrene and necrosis in the strangulated segment but no perforations The strangulated segment was thicker than normal bowel due to hemorrhage in the bowel wall Necrosis was most marked in the mucosa The animal that died at 9½ days was the only animal that refused to eat *following strangulation Autopsy showed no changes in the strangulated* fistula and death in this experiment was attributed more to inanition than to the experimental procedure Since the time interval was so long this death was not considered to be related to the experimental procedure and this animal was considered to have survived the strangulation procedure

DISCUSSION

These studies confirm previous observations about the value of the Thiry fistula preparation for the study of certain phases of strangulation obstruction[1 2] The ease with which these animals can be handled following strangulation is in marked contrast to the difficulty in animals with the usual strangulation obstruction[3 4 7] The ability of these animals to take food and liquids shortly after strangulation and the absence of excessive vomiting greatly simplifies the fluid and electrolyte balance problems and makes easier the study of other phases of this complex problem Blood loss still occurs into the wall and lumen of the fistula but neither of these seems to be as pronounced as in the usual strangulation obstruction experiments and this phase of the postoperative care is simplified also

fluids for 24 to 48 hr after operation After this fluids and then food were allowed The dogs were kept in metabolism cages for intake output studies

During the first 48 hr after operation a total of 40 ml to 200 ml of *Cl welchii* culture containing $3 \times 10^8$ organisms per ml were injected in divided doses into the fistula in an attempt to provide a continuing high concentration of these organisms in the devascularized segment of bowel Peristalsis of the loop removed a large portion of the injected material so that the amount remaining was far less than that injected The amount injected was selected after studies showed that dogs with a normal gastrointestinal tract could swallow 300 to 600 ml of such cultures without effect

For the 1 or 2 days immediately preceding the second operation the fistula was irrigated with antibiotics cultures of *Cl welchii* or both in an attempt to eliminate all other bacteria from the loop following strangulation

Cultures were obtained from the fistula by injecting and then aspirating saline through a catheter threaded into the fistula Cultures were obtained at intervals between the 2 operations before any antibiotics or cultures were instilled into the fistula, and at the time of the second operation

## RESULTS

Bacteriologic studies of the lumen of the fistula indicated that sterilization of the fistula did not occur regardless of the length of time the fistula was allowed to drain The concentration of bacteria undoubtedly decreased but there was not complete removal of bacteria Temporary sterilization was obtained by irrigation of the fistula with antibiotics but this lasted for only 24 to 48 hr after which the previous bacterial flora returned Repeated irrigations with large quantities of sterile saline also failed to remove the bacteria from the fistula

Bacteriologic examination of the peritoneal fluid after strangulation showed that the 4 organisms most commonly found in the fistula—clostridia coliforms streptococci and staphylococci—were found at one time or an other in the peritoneal fluid of each animal though they might not all be there at one time No differences can be detected between the peritoneal fluid bacteriology of the animals that survived and those that died

Following strangulation the change in color of the strangulated segment was just as dramatic as in past experiments[3,4,7] There was immediate cyanosis and hemorrhage into the bowel wall and mesentery After recovery from anesthesia these animals returned to a relatively normal status much more quickly than did animals with strangulation obstruction of normal bowel This was attributed to the lack of vomiting and distention and the absence of obstruction These animals would eat or drink as soon as water or food was offered Vomiting was infrequent occurring only once in 2 experiments and 2 and 3 times in 2 others It is obvious that fluid and electrolyte loss through vomiting is not a major problem with this type preparation and thus eliminates one of the major difficulties in the therapy of animals with experimental strangulation obstruction

The peritoneal fluid aspirated from these animals was usually of a pink color coagulated on standing and generally had no odor Hemolysis was absent or only of minor extent which is in contrast to previous experiments[7]

# MEASUREMENT OF SEQUESTRATION OF RED CELL MASS IN STRANGULATING INTESTINAL OBSTRUCTION UTILIZING RADIOACTIVE $Cr^{51}$ *

Po Myat Ya, John E. Perry, Jr., Maung Soe Thein and Owen H. Wangensteen

Many workers have measured the amount of blood lost from the general circulation in strangulating intestinal obstructions. Scott and Wangensteen[1] compared the weight of the strangulated intestinal segment and its contents and the weight of the peritoneal fluid with that of a similar length of the normal intestine to measure the blood loss into the strangulated loop and the peritoneal fluid. Foster and Hausler[2] placed the strangulated loop in a thin rubber bag and calculated the blood loss from the gain in weight of the rubber bag and its contents with that of comparable controls. Aird[3] determined the plasma volume by the vital red method before strangulation and again when the animal was preterminal. He calculated the blood loss from the difference between these two. It was generally agreed from these experiments that blood loss is the most important lethal factor in long loop strangulating venous obstructions.

The introduction of $Cr^{51}$ labelling of red cells has provided an accurate technique for measurement of red cell mass under various conditions. The following study was undertaken to determine the distribution of red cells in the strangulated segment, the peritoneal fluid and the other viscera in experimental strangulating obstructions.

## METHOD

Mongrel dogs weighing 6 to 15 kg were utilized. The red cell volume was calculated at the time of initial tagging of the red cells. The closed method of red cell volume determination by $Cr^{51}$ using ascorbic acid as a reducing agent for sodium chromate to block plasma radioactivity from entering fresh red cells as described by Read[4] was used throughout the experiment. Heparin was used as an anticoagulant in tagging and drawing of the samples. At the time of injection of the $Cr^{51}$ tagged red cells 0.5 to 1 ml of epinephrine was added to insure thorough mixing of the tagged red cells with the cells of the rest of the body.[5] The femoral vein was exposed by a small incision for drawing blood and reinjection of $Cr^{51}$ tagged blood. The sample taken 15 min after injection was drawn from the opposite femoral vein for the calculation of red cell volume. A minimum of 3 days was allowed for the plasma radioactivity to decline to negligible amounts. Animals were fasted for 24 hr before the strangulation of the intestine. Lengths of intestine varying from 1 to 4 ft were chosen for the experiment. To insure uniformity, the terminal ileum 1 ft proximal to the ileocecal junction was always taken as the distal point of the closed loop and measurements were made from this point. Binding tape was used to occlude the lumen of the bowel at the ends of the segment chosen for strangulation. A window was first made in the mesentery to the bowel at the ends of the

From the Department of Surgery, University of Minnesota Medical School, Minneapolis 14, Minn. Supported by U.S.P.H.S. Supplement RG 1028 (C9), Jay and Rose Phillips Fund, Arthur and Stella Sanford Fund.

The failure to achieve sterilization of the fistula in these long term experiments should settle the question of whether a loop removed from the continuity of the gastrointestinal tract can or cannot sterilize itself

The survival of the majority of animals in this series would indicate that venous strangulation of a segment of bowel out of continuity with the remainder of the gastrointestinal tract is not uniformly fatal This is another step in eliminating degenerating tissue alone as a possible source of some toxic substance in strangulation obstruction Even when the segment is irrigated with pure cultures of *Cl welchii* the result is not uniformly fatal The differences between the animals that survived and those that died is not apparent and has suggested a future course of investigation

Harper and Blain[6] studied the toxemia of intestinal obstruction by utilizing isolated obstructed loops They correlated the toxemia with the presence of distention and bacteria in these loops Their conclusions that an abundant bacterial flora was compatible with life in the absence of distention and that distention was a pre requisite to infection of the intestinal wall by the normal intestinal flora would appear to be confirmed by these experiments which have the added factor of vascular damage to the bowel wall Distention did not occur in these experiments because of the rapid discharge of any fluid or gas formed in the Thiry fistula The additional role that might be played by distention of the Thiry fistula remains to be investigated

The important role of bacteria in strangulation obstruction has been shown by studies which utilized antibiotics to preserve viability in strangulated obstructed bowel[3, 4] Survival in the current experiments may be due to the absence of some other factors which are present in strangulation obstruction These studies are continuing in an attempt to determine what other factors are necessary to achieve a lethal result

SUMMARY

1 The Thiry fistula technique has been applied to another phase of the problem of strangulation obstruction

2 Venous strangulation of a 30 cm segment of a Thiry fistula is not necessarily fatal even when the fistula has been irrigated with a pure culture of *Cl welchii*

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*Table 1 Red Blood Cells Sequestered in Strangulated Loop and Peritoneal Fluid in Closed Loop Venous Strangulations*

| NO OF DOGS | LENGTH OF THE LOOP STRANGULATED | PER CENT OF TOTAL INTESTINE STRANGULATED MEAN | RANGE | SURVIVAL MEAN | RANGE | SEQUESTERED R B C IN STRANGULATED BOWEL AND PERITONEAL FLUID MEAN | RANGE |
|---|---|---|---|---|---|---|---|
| 4 | 1 ft | 9% | 8–10 | 19 hr | 15–23 hr | 35 | 20–49 |
| 3 | 2 ft | 21 | 20–22 | 11 hr | 8–14 hr | 44 | 33–56% |
| 1 | 3 ft | 31% | | 6 hr | | 42% | |
| 2 | 4 ft | 37% | 31–42 | 9 hr | 8–10 hr | 61% | 57–66% |

*Table 2 Distribution of $Cr^{51}$ Labelled Red Blood Cells in Animals Dying of Closed Loop Venous Strangulations*

| DOG | LENGTH OF STRANGULATED LOOP IN FEET | RED CELLS IN ABDOMINAL VISCERA LUNGS | RED CELLS IN STRANGULATED LOOP & PERITONEAL FLUID | % RED CELLS IN REMAINDER OF THE BODY |
|---|---|---|---|---|
| 68* | 0 | 36% | 0 | 64% |
| 41* | 0 | 30% | 0 | 70% |
| 40 | 1 | 19% | 20% | 61% |
| 57 | 1 | 21% | 31% | 48% |
| 15 | 1 | 20% | 40% | 40% |
| 54 | 2 | 14% | 45% | 41% |
| 10 | 3 | 34% | 42% | 24% |
| 37 | 4 | 27% | 57% | 16% |
| 58 | 4 | 17% | 66% | 17% |

Control Dog Killed by Electrocution
* Control Dog Killed with Pentobarbital

general circulation With the longer loops as much as 61 per cent of the red cells is lost into the strangulated loops and peritoneal cavity This leaves little room to doubt the importance of blood loss as a major cause of death in closed loop venous strangulating intestinal obstructions

The distribution of red cells in the viscera the strangulated loop and the peritoneal fluid of these animals are shown in Table 2 as compared with two control dogs It could be seen that in animals dying of venous strangulations of the intestine there is no abnormal pooling of red cells in the viscera to account for the production of symptoms of shock and death

The results with the neomycin group are arranged in Table 3 There was correlation of decreased loss of blood with increased survival time in dogs with the shorter loops With the longer loops survival time was as short and the total sequestered red cells was essentially the same as in the previous group

DISCUSSION

This work confirms the work of previous authors that death in venous strangulating intestinal obstructions can occur rapidly from the simple

loop selected. The vessels running adjacent and parallel to the bowel were cut and ligated. The binding tape was passed through this window around the wall of the bowel and tied. One foot of intestine proximal to the loop was measured off and marked with seromuscular stitches of silk and was used as a measure for the whole length of the intestine at the autopsy. The venous supply of the loop was dissected out and cut between ligatures. Animals were allowed to die without any general supportive measures. A sample of blood was drawn before the death of the animal from which was calculated the red cell mass in the abdominal viscera and lungs at the time of death. Autopsy was performed immediately after the death of the animal. The strangulated loop, the normal intestine, abdominal viscera (kidneys, liver, stomach, spleen, colon) and the lungs were taken out after ligating the blood supply of each to prevent spillage of blood. Peritoneal fluid was first taken out with a large syringe, the peritoneal cavity was then washed several times with normal saline and the washings added to the peritoneal fluid. This procedure was done after the strangulated segment was taken out and before the removal of the abdominal viscera. Each organ was placed in a separate beaker, cut into smaller pieces, treated with 10 per cent NaOH solution overnight. Each viscus was homogenized by mixing in a Waring Blender and by heating in a water bath. Aliquot samples were then obtained for counting in a well type scintillation counter. The red cell mass in the strangulated loop and the peritoneal fluid was calculated from the sample of the blood taken at the time of strangulation. The red cell mass in the viscera was calculated from the sample of the blood taken before the death of the animal.

Two groups of dogs were studied. The first group consisted of 10 animals with closed loop venous strangulations as described above. In the second group of 9 dogs 1.5 gm. of neomycin was instilled into the loop for each foot of bowel strangulated. Three dogs in Group I died before the blood sample could be drawn and the red cell mass determination for the abdominal viscera and lungs were omitted in these 3.

RESULTS

Observations at autopsy were essentially the same as described by previous workers. The strangulated loop was of dark mahogany color, thickened and distended with thick foul smelling bloody material. It was found to be ruptured in 3 out of 4 dogs with 1 ft. loops in Group I. The peritoneum contained from a few ml. to about 250 ml. of thin dark foul smelling serosanguinous fluid. The peritoneal fluid was increased in cases of shorter loop strangulations with longer survival time.

In the animals with neomycin instilled into the loop, the foul smell so characteristic of the former group was absent from the strangulated gut contents and the peritoneal fluid. In 2 out of 3 dogs with 1 ft. loops in the neomycin group there was general peritonitis from the rupture of the proximal nonstrangulated bowel and death may have been due to generalized peritonitis.

The red cell mass sequestered in the strangulated segment and peritoneal fluid in the first group is shown in Table 1. It was found that even though the survival time was longest with the shorter loops, still there was a considerable percentage of the total red cells (mean 35 per cent) lost from the

*Table 1 Red Blood Cells Sequestered in Strangulated Loop and Peritoneal Fluid in Closed Loop Venous Strangulations*

| NO OF DOGS | LENGTH OF THE LOOP STRANGULATED | PER CENT OF TOTAL INTESTINE STRANGULATED | | SURVIVAL | | SEQUESTERED R B C IN STRANGULATED BOWEL AND PERITONEAL FLUID | |
|---|---|---|---|---|---|---|---|
| | | MEAN | RANGE | MEAN | RANGE | MEAN | RANGE |
| 4 | 1 ft | 9 | 8-10 | 19 hr | 13-23 hr | 35 | 20-49 |
| 3 | 2 ft | 21 | 20-22 | 11 hr | 8-14 hr | 41% | 35-56 |
| 1 | 3 ft | 31% | | 6 hr | | 42% | |
| 2 | 4 ft | 37 | 31-42 | [illegible] hr | 8-10 hr | 61 | 57-66% |

*Table 2 Distribution of Cr[51] Labelled Red Blood Cells in Animals Dying of Closed Loop Venous Strangulations*

| DOG | LENGTH OF STRANGULATED LOOP IN FEET | % RED CELLS IN ABDOMINAL VISCERA LUNGS | % RED CELLS IN STRANGULATED LOOP & PERITONEAL FLUID | % RED CELLS IN REMAINDER OF THE BODY |
|---|---|---|---|---|
| 68 | 0 | 36% | 0 | 64% |
| 41** | 0 | 30% | 0 | 70% |
| 40 | 1 | 19% | 20% | 61% |
| 57 | 1 | 21% | 31% | 48% |
| 15 | 1 | 20% | 40% | 40% |
| 54 | 2 | 14% | 45% | 41% |
| 10 | 3 | 34% | 42% | 21% |
| 37 | 4 | 27 | 57% | 16% |
| 58 | 4 | 17% | 66% | 17% |

* Control Dog Killed by Electrocution
Control Dog Killed with Pentobarbital

general circulation With the longer loops as much as 61 per cent of the red cells is lost into the strangulated loops and peritoneal cavity This leaves little room to doubt the importance of blood loss as a major cause of death in closed loop venous strangulating intestinal obstructions

The distribution of red cells in the viscera the strangulated loop and the peritoneal fluid of these animals are shown in Table 2 as compared with two control dogs It could be seen that in animals dying of venous strangulations of the intestine there is no abnormal pooling of red cells in the viscera to account for the production of symptoms of shock and death

The results with the neomycin group are arranged in Table 3 There was correlation of decreased loss of blood with increased survival time in dogs with the shorter loops With the longer loops survival time was as short and the total sequestered red cells was essentially the same as in the previous group

DISCUSSION

This work confirms the work of previous authors that death in venous strangulating intestinal obstructions can occur rapidly from the simple

*Table 3 Red Blood Cells Sequestered in Strangulated Loop and Peritoneal Fluid in Closed Loop Venous Strangulation of the Intestine (Neomycin Instilled into the Loop)*

| NO. OF DOGS | LENGTH OF THE LOOP STRANGULATED | % OF TOTAL INTESTINE STRANGULATED MEAN | RANGE | SURVIVAL MEAN | RANGE | SEQUESTERED RED CELLS IN STRANGULATED BOWEL AND PERITONEAL FLUID MEAN | RANGE |
|---|---|---|---|---|---|---|---|
| 3 | 1 ft | 10% | 10–11% | 110 hr | 84–148 hr | 20% | 10–33% |
| 3 | 2 ft | 23% | 18–29% | 32 hr | 9–50 hr | 46% | 42–51% |
| 1 | 3 ft | 27% | | 7 hr | | 58% | |
| 2 | 4 ft | 33% | 30–36% | 8 hr | 8–9 hr | 63% | 53–73% |

cause of blood loss alone. Even with moderately short loops comprising about 10 per cent of the total length, as much as 49 per cent of the total red cell mass of the body could be lost into the strangulated loop from the circulation. Our results show even greater losses of red cells in short loops than had been reported by other workers.[1,2,3]

In the series of animals treated by instillation of neomycin into the loop the longer survival time occurs with decreased loss of red cells into the loop. This decreased tendency to bleed into the loop has been noted by others in dealing with the open loop venous strangulations.[6,7] It raises the question again as to whether decreased blood loss into the loop may not be a significant factor in prolonging the survival in animals with experimental venous strangulations in which neomycin has been instilled into the loop. These experiments in no way detract from the observation of others suggesting suppression of bacterial factor in the prolongation of survival of the animals with venous strangulating obstructions by antibiotics.[6,7,8,9,10]

Histologic examination of sections from those with and without neomycin in the strangulated loop at death offered no explanation for the decreased bleeding in the neomycin group, although others have described a preservation of near normal histologic appearance of the bowel wall in the open loops with venous strangulation protected by antibiotics.[6,7]

SUMMARY

The distribution of $Cr^{51}$ labelled red blood cells in animals dying of closed loop venous obstruction of intestine of varying lengths has been studied. Survival time varies inversely with the length of the strangulated segment and the principal cause of death is loss of blood even with 1 ft loops. From the distribution of the red cell mass in the viscera at the time of death there is no evidence to suggest any pooling of the blood in the viscera. With neomycin instilled into the strangulated loop decreased cell loss occurs concomitantly with increased survival in animals with shorter loops, but red cell loss as well as survival is almost the same in the control and neomycin groups with longer loops.

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# SURGICAL JAUNDICE EXPERIMENTAL EVIDENCE AGAINST THE REGURGITATION THEORY *

HORACE D RITCHIE JOHN H GRINDLAY AND JESSE L BOLLMAN

More than 150 years have passed since the first ligation of the common bile duct was carried out by Saunders He observed that bile pigments promptly accumulated in the lymph of the thoracic duct Since then a great deal of work has been done and much has been written on this phenomenon but opinion is still divided as to its significance

It has been suggested that the phenomenon may be due to regurgitation of bile from the biliary tree into the liver lymph which would then be carried to the thoracic duct and so to the blood itself and that such is the mechanism of surgical jaundice

Evidence for this view is based mainly on observations of the bilirubin content of thoracic duct lymph from anesthetized dogs within the first few hours after biliary obstruction Only acute studies are possible with such preparations and it is necessary first to remove the gallbladder

We have attempted to simulate more closely actual clinical conditions by producing acute repeated and chronic obstruction of the bile ducts in conscious intact or cholecystectomized dogs The lymph coming from the liver in these conditions has been examined directly for evidence of regurgitation

## METHOD

Fifty healthy mongrel dogs weighing 8 to 12 kg were used in this study and in these animals liver lymph fistulae were established[1] The experi

* From the Mayo Foundation and Mayo Clinic The Mayo Foundation Rochester Minn is a part of the Graduate School of the University of Minnesota

*Table 3 Red Blood Cells Sequestered in Strangulated Loop and Peritoneal Fluid in Closed Loop Venous Strangulation of the Intestine (Neomycin Instilled into the Loop)*

| NO OF DOGS | LENGTH OF THE LOOP STRANGULATED | % OF TOTAL INTESTINE STRANGULATED | | SURVIVAL | | SEQUESTERED RED CELLS IN STRANGULATED BOWEL AND PERITONEAL FLUID | |
|---|---|---|---|---|---|---|---|
| | | MEAN | RANGE | MEAN | RANGE | MEAN | RANGE |
| 3 | 1 ft | 10% | 10–11% | 110 hr | 84–148 hr | 20% | 10–33% |
| 3 | 2 ft | 23% | 18–29% | 32 hr | 9–50 hr | 46% | 42–51% |
| 1 | 3 ft | 27% | | 7 hr | | 58% | |
| 2 | 4 ft | 33% | 30–36% | 8 hr | 8–9 hr | 63% | 53–73% |

cause of blood loss alone Even with moderately short loops comprising about 10 per cent of the total length as much as 49 per cent of the total red cell mass of the body could be lost into the strangulated loop from the circulation Our results show even greater losses of red cells in short loops than had been reported by other workers [1, 2, 3]

In the series of animals treated by instillation of neomycin into the loop the longer survival time occurs with decreased loss of red cells into the loop This decreased tendency to bleed into the loop has been noted by others in dealing with the open loop venous strangulations [6, 7] It raises the question again as to whether decreased blood loss into the loop may not be a significant factor in prolonging the survival in animals with experimental venous strangulations in which neomycin has been instilled into the loop These experiments in no way detract from the observation of others suggesting suppression of bacterial factor in the prolongation of survival of the animals with venous strangulating obstructions by antibiotics [6, 7, 8, 9, 10]

Histologic examination of sections from those with and without neomycin in the strangulated loop at death offered no explanation for the decreased bleeding in the neomycin group although others have described a preservation of near normal histologic appearance of the bowel wall in the open loops with venous strangulation protected by antibiotics [6, 7]

SUMMARY

The distribution of $Cr^{51}$ labelled red blood cells in animals dying of closed loop venous obstruction of intestine of varying lengths has been studied Survival time varies inversely with the length of the strangulated segment and the principal cause of death is loss of blood even with 1 ft loops From the distribution of the red cell mass in the viscera at the time of death there is no evidence to suggest any pooling of the blood in the viscera With neomycin instilled into the strangulated loop decreased cell loss occurs concomitantly with increased survival in animals with shorter loops but red cell loss as well as survival is almost the same in the control and neomycin groups with longer loops

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# COMMON DUCT PRESSURES FOLLOWING SPHINCTEROTOMY*

W H Brown T Earley and B Eiseman

The physiologic rationale for sphincterotomy in the treatment of chronic pancreatitis depends upon the prevention of biliary reflux into the pancreatic duct by decreasing the intraluminal choledochal pressure[1,2,3] Although common duct pressures occasionally have been measured immediately after sphincterotomy[4] there have been no such studies covering a prolonged period following this operation

This is a report of the emptying pressures in the common duct of dogs over a prolonged period following sphincterotomy and thus is a measurement of the efficacy of this operation in preventing biliary regurgitation into the pancreas

## METHOD

Intraductile free flow pressures have been determined in a series of 18 dogs via a common duct T tube exteriorized through a right upper quadrant stab wound

Grindlay s technique[5] for maintaining the catheter in place was found to be excellent for pressure measurements in the immediate postoperative period but the animals in this study invariably extricated the catheters within 2 to 4 wks Fixation of the T tubes within a windowed and bivalved plaster cast resulted in respiratory complications in a majority of the dogs so treated In 18 animals the T tube was fitted intraperitoneally to a plastic cannula which penetrated the abdominal wall and was firmly fixed by an adjustable flange that screwed over the cannula and rested on the skin (Fig 1)

With the unanesthetized fasting animal lying on his left side physiologic saline was slowly (5 ml/min) injected into the T tube and pressures recorded on a spinal manometer inserted into the system via a glass Y tube The level of the porta hepatis was taken as the base line The pressure within the system gradually increased as fluid was injected into the system

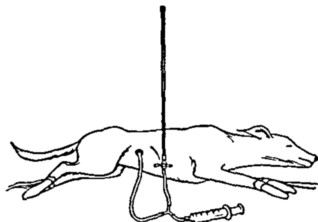


Fig 1 Diagram of method for determining common duct free flow pressure

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mon bile duct in the normal dog most of the bilirubin which accumulates in the blood stream has been discharged into it directly from the biliary tree during its passage through the liver

If these findings could be regarded as valid for man they would suggest that regurgitation of bile into the liver lymph during biliary obstruction could occur only if the gallbladder had been removed or was nonfunctioning and that this phase would be over about 24 hr after the obstruction had been established Its contribution to the ultimate jaundice would therefore be a minor one

Moreover, should the blockage relieve itself and as in obstruction by calculus recur soon afterward or if a functioning gallbladder were present, as in many neoplastic obstructive conditions there would appear to be little evidence that regurgitation into the lymph could play any part in the production of the ensuing jaundice


SUMMARY

1 Acute repeated and chronic obstruction of the common bile duct has been produced in 50 conscious dogs The concentrations of bilirubin in the lymph coming from the liver and in the blood of these animals have been measured at intervals for periods of up to 4 days The data have been scrutinized for evidence of regurgitation of bilirubin into the liver lymph

2 Evidence of such regurgitation was found only during the first 24 hr of a first bout of obstruction in the cholecystectomized animals

3 No evidence of regurgitation was found (a) after the first 24 hr in the cholecystectomized animals (b) during any subsequent biliary obstruction in the cholecystectomized animals (c) in acute obstruction when the gallbladder was present or (d) during chronic obstruction in animals with or without a gallbladder

4 Sulfobromophthalein studies have confirmed these findings


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sure of 30 cm of saline prior to operation In a number of animals resistance to flow was already perceptible at the time of laparotomy closure Twenty four hr postoperatively the free flow pressures were approximately 30 per cent below the preoperative levels and usually remained depressed for the next 5 days Thereafter the pressure gradually returned to preoperative levels and remained so for the balance of the experiment Representative experiments are illustrated in Figure 2 Neither double cut sphincterotomy nor leaving the gallbladder intact altered the basic free flow pressure response

An increase in free flow pressure following the instillation of hydrochloric acid was noted in each case prior to sphincterotomy a pattern which disappeared only for 24 to 48 hr following operation Thereafter acid stimulation produced a progressively greater response which within a week resulted in a resistance to flow equal to that found preoperatively

Injection of saline into the T tube at the time the animals were sacrificed 6 to 8 wks following sphincterotomy produced a thin high trajectory stream from the common duct into the duodenum similar to that found prior to sphincterotomy and in contrast to the wide diameter gush noted immediately following sphincter section

Microscopic examination of the sphincters revealed fibrous union between the musculature surrounding the duct at the site of its previous section regardless of the type of sphincterotomy previously performed

## DISCUSSION

These experiments indicate that sphincterotomy diminished the choledochal free flow pressure in the dog for only a few days following operation They further indicate that the rise in emptying pressure resulting from the injection of hydrochloric acid into the duodenum that is said[3, 6] to be indicative of sphincter action persists to a significant though diminished degree following section of the sphincter and returns to preoperative levels within a week They further suggest that choledochal free flow pressures are not primarily regulated by sphincter action or if so governed are not permanently affected by sphincterotomy

Factors other than sphincter of Oddi contraction that might alter choledochal emptying pressure include the pinchcock action of the duodenal wall as the duct passes through it obliquely or the contractile action of the circular smooth muscle fibers in the wall of the common duct proximal to its entrance into the duodenum Duodenal pressures are not transmitted directly to the common duct[6]

The immediate rise in free flow pressure after operation may be due to wound edema at the site of sphincterotomy but this would be expected to subside within a few days Healing of the singly or doubly sectioned sphincter on the other hand may account for the late return of sphincter tone as the encircling muscle fibers are joined by fibrous scar

Whatever the physiologic explanation may be sphincterotomy as here performed does not permanently affect the free flow pressures of the common bile duct in the dog nor does it permanently alter the response of such pressure to hydrochloric acid instillation into the duodenum These experiments therefore throw serious doubt on the physiologic rationale of sphincterotomy in management of chronic pancreatitis

and then fell suddenly at the moment it emptied into the duodenum The pressure required to overcome the resistance to flow has been called the free flow pressure The mean of 10 consecutive measurements performed at 1 to 3 min intervals has been used for each experiment, and has varied routinely less than 5 cm of saline Control free flow pressures were recorded weekly for 3 wks after insertion of the T tube and cholecystectomy Pressures were then recorded immediately prior to sphincterotomy at the time of opening the duodenum after sphincter section immediately following duodenal closure at the time of laparotomy closure daily for 7 days and finally at weekly intervals 6 times thereafter Following each such pressure measurement 8 ml of 1/N HCl was injected into the duodenum via the T tube and free flow pressures measured immediately thereafter

Extreme care was taken in the performance of the transduodenal sphincterotomy to cut the intramural muscle fibers as well as those of the sphincter and to assure that an uninterrupted stream flowed into the duodenum This necessitated eversion of the ampulla and proximal section of the sphincter fibers for approximately 2 0 cm

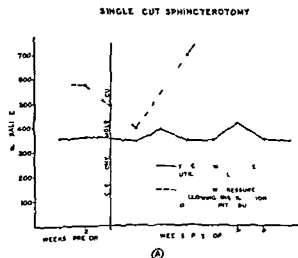
In 6 animals the sphincter of Oddi was sectioned in 2 places at 90 angles to each other (6 and 3 o clock) in an effort to produce biliary incontinence

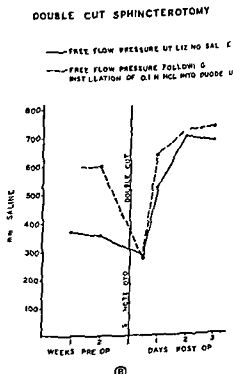
At the end of 8 wks all animals were sacrificed and the common duct and sphincter of Oddi examined grossly and microscopically to assure complete severance of all sphincter fibers and to find evidence of healing

## RESULTS

Immediately following sphincterotomy the patulent sphincter allowed a free flow from the common duct into the duodenum and there was but an imperceptible resistance to injection compared to the mean free flow pres

Fig 2 Representative experiments of common duct free flow pressures showing response to hydrochloric acid injection before and after sphincterotomy a) Single cut sphincterotomy b) Double cut sphincterotomy



patients who survived for 2 mos or more after operation have been selected for the present report and certain aspects of sodium metabolism as it relates to ascites will be stressed

METHOD

Following an initial 3 to 5 day period during which sodium intake was normal a high protein high caloric low sodium diet (10 mEq /day) was instituted Some patients stabilized their ascites on this program alone others required occasional paracenteses or courses of ion exchange resins, but all showed residual ascites at the time of operation Low serum albumin levels were corrected by administration of intravenous albumin All patients were maintained on complete water electrolyte and nitrogen balance for periods of 1 to 8 wks preoperatively and 3 to 8 wks postoperatively End to side or side to side portacaval shunts were performed following which sodium intake was maintained at a low level as long as urinary sodium output remained low Thereafter a more liberal sodium intake was occasionally permitted, but intake was limited to an amount less than that being excreted daily in the urine The usual laboratory tests of liver function were obtained at frequent intervals throughout the preoperative and post operative periods The data on sodium intake and urinary sodium output originally determined and calculated on a daily basis are presented here as averages over weekly periods

RESULTS

The 7 patients presented fall into 2 distinct groups Four (Group 2) have survived 2 to 6 yrs after portacaval shunts, and none has shown re accumulation of ascites The remaining 3 patients (Group 2) died 2 3 and 18 mos after operation and all showed recurrent ascites As far as could be determined preoperatively the 2 groups were comparable in severity of liver damage and clinical condition

**Serum Sodium levels** The mean values of the preoperative serum sodium levels were essentially the same in both groups 134 2 and 135 5 mEq/L in Groups 1 and 2 respectively However Group 1 patients showed a rise in the postoperative serum sodium concentration to a mean value of 138 6 mEq whereas Group 2 patients showed a decreasing serum sodium level with a mean postoperative value of 129 2 mEq

**Urinary Sodium excretion** The mean values of the preoperative urinary sodium values were approximately the same in both groups 2 3 mEq /day in Group 1 and 2 7 mEq /day in Group 2 There was a marked difference however in the postoperative period between the 2 groups Urinary sodium excretion for Group 2 patients who subsequently re accumulated ascites and died remained at a low level throughout the entire postoperative period the mean urinary sodium values being 1 9 mEq /day The Group 1 patients however demonstrated a consistent increase in urinary sodium levels in the postoperative period usually starting by at least the 3rd or 4th wk The mean daily urinary sodium level was 3 8 mEq during the first postoperative week 9 7 mEq for the second week 21 8 mEq for the third week and 37 4 mEq for the fourth week Generally no increase in sodium intake above 10 mEq /day was permitted until the urinary output reached 15–20 mEq daily

SUMMARY

1 Free flow pressures within the common duct have been measured in a series of 18 dogs at representative periods before and for prolonged periods following both single and double section of the sphincter of Oddi

2 Resistance to biliary emptying into the duodenum as thus measured returns to pre sphincterotomy level almost immediately following operation and remains elevated for a prolonged period thereafter

3 The free flow pressure response to intraduodenal hydrochloric acid is but temporarily reduced following complete section of the sphincter

4 It is concluded that sphincterotomy in dogs is not permanently effective in reducing the resistance to flow from the common duct into the duodenum

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# CHANGES IN SODIUM METABOLISM FOLLOWING PORTACAVAL SHUNTS IN PATIENTS WITH CIRRHOSIS AND ASCITES*

KEITH REEMTSMA ROBERT E CARLSON DAVID V HABIF AND HAROLD G BARKER

Patients with cirrhosis portal hypertension and ascites who undergo portacaval shunt operations frequently show clearing of ascites in the postoperative period The majority of such patients have minimal ascites which responds quickly to mercurial diuretics and therefore require no special preoperative care However cirrhotics who have long standing severe ascites which is unresponsive to mercurial diuretics constitute a special group of patients with whom this study is concerned The 7 most completely studied

*From the Department of Surgery Columbia University College of Physicians and Surgeons and the Surgical Service Presbyterian Hospital New York Supported in part by U S Public Health Grant RG 2926 (C-4)

5 Failure to start excreting at least 10 mEq of sodium daily by the third postoperative week was a poor prognostic sign

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# A COMPARISON OF LIVER FUNCTION FOLLOWING ECK FISTULA AND PORTACAVAL TRANSPOSITION*

WILLIAM SILEN DEAN L MAWDSLEY WILLIAM L WEIRICH HAROLD A HARPER AND H J McCORKLE

It has been observed that the function of the liver is impaired in dogs in which an Eck fistula has been surgically produced [1 2 3] The classical syndrome of meat intoxication may easily be induced in dogs with Eck fistulas [4] but as recently shown in this laboratory,[5] this symptom complex cannot be elicited in animals with surgically produced transposition of the portal vein and vena cava even after prolonged administration of raw meat A comparative study of the function of the liver before and at several intervals after each of these 2 operations was therefore undertaken in an effort to discover some of the causes for the apparent differences in function that were observed

## METHOD

All surgical procedures were carried out with the use of intravenous sodium nembutal anesthesia and strict aseptic precautions In 6 dogs (Group 1) an Eck fistula was produced through a right thoracoabdominal incision Six dogs (Group 2) underwent the operation of transposition of the portal

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**Liver Function Studies** No consistent pattern emerged from analysis of pre and postoperative serum albumin levels cephalin flocculation tests alkaline phosphatase determinations or bromsulphonthalein excretion values There were no marked differences between Group 1 and Group 2 patients and no consistent differences in individual patients between preoperative and postoperative periods

## DISCUSSION

This study demonstrates that in a group of patients with cirrhosis and ascites subjected to portacaval shunts the postoperative changes in sodium metabolism are closely correlated with the clinical course of the patient including the disappearance or recurrence of ascites

The exact mechanisms responsible for the disappearance of ascites following portacaval shunts are obscure The relationship between extrahepatic portal pressure and ascites formation has not been clearly defined although experimental evidence suggests that they may not be closely correlated The delayed onset of urinary sodium diuresis for 3 wks or more as we and others[1] have seen in some patients after reduction in portal pressure is not easily explained on the mechanical effect of reduced portal pressure, although sodium retention for perhaps 10 to 14 days might be expected to follow a procedure of the magnitude of a portacaval shunt One further point against the mechanical theory is the lack of correlation between presence or absence of ascites and the degree of portal hypertension [2] A second possible mechanism concerns the role of plasma proteins particularly albumin but this study and others[3] suggest that serum albumin levels are not correlated with ascites formation

During recent years increasing attention has been focused on sodium metabolism in ascites [3, 4] and its relationships to adrenal and pituitary function [5, 6, 7] It is possible that the disappearance of ascites following portacaval shunts may be related to a humoral mechanism influencing sodium distribution and excretion The liver may play a detoxifying or other role in such a system and it is possible that the relief of portal hypertension may lead to subtle improvement in this particular liver function not detectable by the ordinary tests That this may be a factor is suggested by the observation that serum sodium levels and urinary sodium excretion are consistently elevated in patients who show no recurrence of ascites following portacaval shunts

## SUMMARY

1 Seven selected patients with cirrhosis portal hypertension and severe ascites are reported They were studied by balance techniques in the surgical metabolism unit for 4 to 8 wks before and 3 to 8 wks after portacaval shunting

2 The group of 4 patients who have shown no recurrence of ascites for 2 to 6 yrs following shunts all demonstrated rising serum sodium levels and increasing urinary sodium output in the postoperative period

3 A group of 3 patients who demonstrated recurrent ascites and subsequently died showed persisting or increasingly pronounced hyponatremia and continued low urinary sodium levels in the postoperative period

4 Liver function studies and serum albumin levels could not be correlated with the clinical course

was found to be occluded at autopsy although the other was still patent As a result this animal had an Eck fistula together with obstruction of the return of venous blood from the kidneys and lower extremities With respect to liver function clinical signs and symptoms and relation of liver weight to body weight the findings in this dog were typically those of an animal with an Eck fistula Microscopic examination of the kidneys revealed no abnormality

There was a rather marked difference in the general condition of the 2 groups of dogs as the experiment progressed All of the animals with Eck fistulas lost weight and had a tendency to anorexia lassitude, and polydipsia The mean weight loss of these dogs was 2 34 kg over a 3 mo period In contrast most of the dogs with portacaval transposition either maintained a constant weight or gained small amounts of weight Furthermore these dogs maintained normal activity and appetite and none developed the syndrome of lassitude anorexia and polydipsia observed in the animals with Eck fistulas

The total circulating plasma albumin decreased in every dog with an Eck fistula the mean loss being 19 9 gm On the other hand the total circulating albumin increased (with one exception) in all animals with portacaval transposition the mean gain being 13 8 gm over a 3 mo period The globulin content of the plasma and the results of the thymol turbidity test remained normal in all of the animals in both groups

Clearance of bromsulfalein decreased in every animal with an Eck fistula but rose markedly in every dog with a portacaval transposition (Fig 2a)

In both groups of dogs tolerance to orally administered ammonium lactate was diminished following operation (Fig 2b) This was much more evident however in the dogs with Eck fistulas The mean values for the peak levels of ammonia nitrogen attained in the tolerance tests were 4 56 $\mu$g /ml for the animals in Group 1 and 3 97 $\mu$g /ml for those in Group 2 *despite the* fact that those with Eck fistulas received only one half the dose of ammonium lactate given to the animals with portacaval transposition

The mean fasting level of ammonia nitrogen in the blood of all of these dogs increased within 3 mo after operation (Fig 2b) The increase was slight in the animals with portacaval transposition but very marked in those with Eck fistulas These observations on ammonia tolerance suggest that the response of the 2 groups of experimental animals *to the feeding* of large quantities of raw meat may be attributed to the differences in the clearance of ammonia that existed in these *animals in accordance* with the adequacy of the blood supply to the livers

The gross appearance of the livers was *normal* in both groups of experimental animals except that the livers of the dogs with an Eck fistula were usually smaller This finding is similar to that of Whipple [1] who described marked atrophy of the liver in dogs *with Eck fistulas* On the other hand the relation between the weights of the livers and the body weights in the dogs with portacaval transposition compared favorably with the normal

Histologic studies of the livers of the animals with Eck fistulas revealed a tendency to the accumulation of fat in the *central* zones of the lobules and a loss of hepatic cells adjacent to the central vein The glycogen content of the cells was also decreased These changes were present in the livers of all of the dogs in Group 1 varying only in degree with respect to a given speci

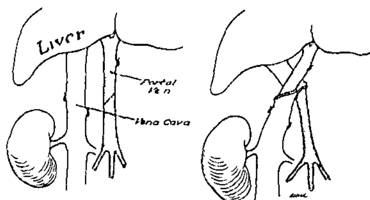



Fig 1 Diagram showing the technique of transposing the portal vein and vena cava

vein and vena cava which was carried out in the manner described by Child[6] (Fig 1) The dogs were kept on the usual kennel rations throughout the experiment

In 4 dogs with Eck fistulas and in 5 dogs with portacaval transpositions studies of liver function were carried out preoperatively and at intervals of 1 wk and 1 2 and 3 mo after operation These studies included measurement of total serum protein as well as albumin and globulin thymol turbidity clearance of bromsulfalein and tolerance to orally administered ammonium lactate The clearance of bromsulfalein was determined by the method of Goodman[7] By extrapolation of the bromsulfalein concentration to zero time the plasma volume of each animal was calculated and the plasma volumes so obtained together with the measured serum albumin concentrations were used to compute the amounts of total circulating protein

Tolerance to orally administered ammonium lactate was determined in the following manner After a sample of blood had been taken from the fasting animal for an estimation of the level of ammonia a test dose of 0 93 gm ammonium lactate/kg of body weight was given to the animals in Group 2 (portacaval transposition), one half that dose was administered in the same way to the animals with Eck fistulas (Group 1) since preliminary studies had shown that these dogs tolerated poorly the larger dose Specimens of blood were drawn at specified intervals after the test dose and the ammonia content of each sample was promptly determined using a modification of the microdiffusion method of Conway[8]

The body weight of each of the animals in the fasting state was recorded each time the laboratory tests were performed At the end of these studies the dogs were sacrificed At autopsy the livers were removed and weighed immediately in the fresh state and specimens of 1 of the kidneys and of 3 different lobes of the livers were obtained for microscopic examination In addition to the usual stains special glycogen (Schiff s stain) and fat (Oil Red O) stains were used to study the liver specimens

## RESULTS

All of the dogs survived the test period The vascular anastomoses between the portal vein and vena cava were found to be patent in all of the animals of both groups with the exception of 1 dog with a portacaval transposition The repair of a tear in the wall of the portal vein at the time of operation had narrowed the lumen of the anastomosis between the distal vena cava and the proximal portal vein and it was this anastomosis which

was found to be occluded at autopsy although the other was still patent As a result this animal had an Eck fistula together with obstruction of the return of venous blood from the kidneys and lower extremities With respect to liver function clinical signs and symptoms and relation of liver weight to body weight the findings in this dog were typically those of an animal with an Eck fistula Microscopic examination of the kidneys revealed no abnormality

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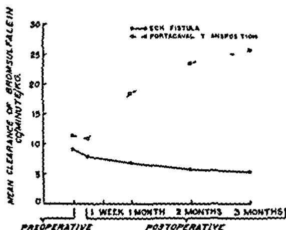



Fig 2 (a) Graph showing the mean values for bromsulfalein clearance for animals with Eck fistulas and those with portacaval transposition

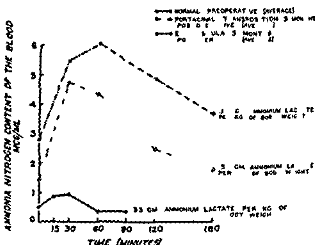



Fig 2 (b) Graph showing ammonia tolerance in dogs with Eck fistulas as compared to normal dogs and those with portacaval transposition

men The livers of the dogs with portacaval transposition were entirely normal and glycogen and fat were present in normal amounts

The kidneys of both groups of animals were normal both grossly and microscopically


SUMMARY

A group of dogs with Eck fistulas and another group with transposition of the portal vein and vena cava were prepared to compare the effects of these 2 operations The animals with Eck fistulas lost weight and their serum albumin levels bromsulfalein clearance and tolerance to ammonium lactate were markedly less than those of normal animals or those with portacaval transposition Histologic examination of the livers of the animals with Eck fistulas revealed fatty infiltration decrease of glycogen and loss of cells in the central zone In contrast all the findings in the dogs with portacaval transposition were very similar to those of normal animals


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# METABOLIC EFFECTS OF TRANSPOSITION ON THE PORTAL VEIN AND INFERIOR VENA CAVA*

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Transposition of the portal vein and inferior cava was first performed in dogs by Child[1] and coworkers in 1953 in a study of liver regeneration. They commented on the frequent occurrence of shock that followed the procedure, a complication since noted by others.[2] This paper is a study of some of the metabolic effects of portacaval transposition with particular emphasis on the cause and avoidance of postoperative shock that so frequently accompanies this operation.

## METHOD

Transposition of the portal vein and inferior vena cava has been performed in a series of 61 adult mongrel dogs anesthetized with intravenous sodium pentobarbital anesthesia (35 to 50 mg./kg. body weight), and artificially respired via an indwelling endotracheal tube. With the animal in the left lateral position a right thoracoabdominal incision was made and the portal vein mobilized from the hilus of the liver to its emergence from the pancreas. The inferior vena cava was similarly mobilized from its entrance into the right lobe of the liver to the right renal vein.

The veins were transected between vascular clamps, transposed and the anastomosis performed with 5-0 arterial silk (Fig. 1). Since occlusion of the portal system in dogs is poorly tolerated for more than 15 min.[3] the distal portal vein was first anastomosed to the proximal cava.

From the outset these animals tolerated the procedure poorly. The operation itself was usually uneventful, requiring a total of 40 to 70 min., and as

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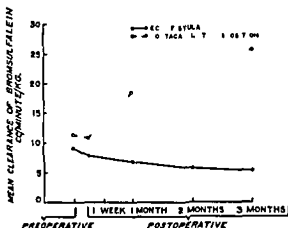



Fig. 2. (a) Graph showing the mean values for bromsulfalein clearance for animals with Eck fistulas and those with portacaval transposition.

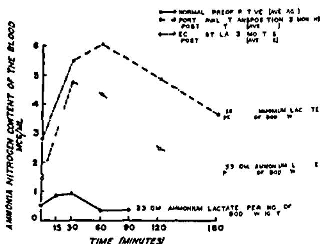



Fig. 2. (b) Graph showing ammonia tolerance in dogs with Eck fistulas as compared to normal dogs and those with portacaval transposition.

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The kidneys of both groups of animals were normal both grossly and microscopically.

## SUMMARY

A group of dogs with Eck fistulas and another group with transposition of the portal vein and vena cava were prepared to compare the effects of these 2 operations. The animals with Eck fistulas lost weight and their serum albumin levels, bromsulfalein clearance, and tolerance to ammonium lactate were markedly less than those of normal animals or those with portacaval transposition. Histologic examination of the livers of the animals with Eck fistulas revealed fatty infiltration, decrease of glycogen, and loss of cells in the central zone. In contrast, all the findings in the dogs with portacaval transposition were very similar to those of normal animals.

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*Table I Summary of Procedures Employed to Avoid Postoperative Shock*

| PROCEDURE | NO DOGS | DIED FROM KNOWN TECHNICAL CAUSE | DIED IN SHOCK | SURVIVED |
|---|---|---|---|---|
| A No Supportive Therapy | 7 | 1 | 3 | 0 |
| B Diminution of deleterious effect of portal venous occlusion | | | | |
| 1 Blood transfusion | 10 | 3 | 6 | 1 |
| 2 Hypothermia | 2 | 0 | 2 | 0 |
| 3 Hypothermia plus clamping thoracic aorta | 1 | 0 | 1 | 0 |
| 4 Anastomosis over siliconized tube | 2 | 0 | 2 | 0 |
| 5 Use of non obstructing anastomosis clamp | 2 | 0 | 2 | 0 |
| 6 By pass shunts (2 Methods) | 5 | 1 | 4 | 0 |
| 7 Splenectomy | 3 | 1 | 2 | 0 |
| 8 Staged procedure avoiding portal occlusion (2 Methods) | 8 | 0 | 8 | 0 |
| C Reversal of order of anastomosis | 2 | 1 | 1 | 0 |
| D Oxygenation of liver | | | | |
| 1 Temporary splenic artery portal vein shunt | 3 | 0 | 3 | 0 |
| 2 Permanent splenic artery portal vein shunt | 2 | 0 | 2 | 0 |
| E Adrenal cortical replacement therapy | | | | |
| 1 Intravenous compound F at operation only | 1 | 0 | 1 | 0 |
| 2 Pre and postoperative cortisone therapy | 16 | 4 | 0 | 12 |
| Totals | 64 | 14 | 37 | 13 |

unobstructed upper extremities In 3 dogs polyethylene tubes were placed in the portal vein and inferior cava and blood pumped via a syringe and 3 way stopcock into the jugular vein during the period of venous occlusion In 2 other temporarily heparinized animals a Sigmamotor pump as currently employed in the pump oxygenator[4] was substituted for the syringes so that continuous perfusion was attained Of the 5 animals in this group 1 expired because of a technical cause and the other 4 succumbed in progressive shock 2 to 12 hr after an otherwise uneventful operative procedure

Performance of the anastomoses in the reverse manner with flow first being resumed from the cava into the liver did not avoid postoperative death from shock in 3 animals

In 8 animals portal venous interruption was entirely avoided by performing a preliminary side to-side portacaval anastomosis In half of these animals a vein graft was interposed end to-end between the portal vein severed above its anastomosis to the vena cava and to the severed cava inferiorly In the other 4 animals a vein graft was placed into the side of the intact distal

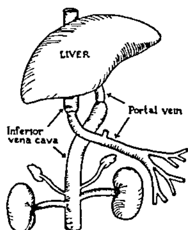



Fig. 1 Diagram of the completed portacaval venous transposition

sociated with minimal blood loss but the animals routinely expired in progressive and uncontrolled shock 2 to 18 hr following operation Of the 64 animals included in this study 51 animals expired in shock during the immediate postoperative period (Table 1) Of these 14 animals expired from a known technical cause and are not further included in the statistical analysis In 8 animals this progressive shock was accompanied by a bloody diarrhea appearing in the immediate postoperative period and which was at first assumed to be due to prolonged portal venous occlusion

The first 7 animals were operated upon without supportive blood transfusions 4 died of technical difficulties and 3 of unexplained shock 2 to 12 hr following operation

In the next 10 animals compatible blood transfusions (mean of 270 ml) were utilized to avoid hypotension All transfusions were given into the upper extremities in order to avoid overloading the lower extremities during temporary venous occlusion Four animals died because of technical difficulties and 5 others expired in the immediate postoperative period in uncontrollable shock and with bloody diarrhea The only survival in this group had a previous splenorenal anastomosis

At this point steps were taken to diminish the time and deleterious effect of portal venous occlusion Hypothermia (83 5 to 84 5°F) was employed in 3 dogs during the procedure and in 1 animal was combined with cross clamping of the thoracic aorta during the period of portal venous occlusion Despite these measures all 3 animals died in postoperative shock Portal occlusion time was reduced to 3 to 5 min and in 2 dogs anastomoses were performed over siliconized polyethylene tubes so that portal flow was interrupted less than 2 min In 2 other animals vascular occlusion time was further minimized by employment of a vascular clamp of our own design which allows continued blood flow during the period of vascular suture In all of these cases however the animals died in postoperative shock

In order to further reduce vascular pooling within the portal bed during venous occlusion splenectomy was performed on 3 dogs prior to performing transposition of the portal vein and inferior cava One animal died of a technical difficulty but the other 2 expired after operation in uncontrollable shock

Attempts were then made during the period of venous occlusion to pump blood from below the point of caval occlusion in the lower extremities to the

It seems evident that the shock occurring following portacaval transposition is due to hypoadrenalism since all animals died when not given supportive adrenal cortical replacement therapy and none died from unexplained causes when such replacement was practiced The combination of the stress of operation plus the hepatic inactivation of the adrenal corticoids proved lethal to the unsupported animal

Bloody diarrhea is a known clinical manifestation of adrenal cortical insufficiency[9] and its occurrence in a number of the animals dying in shock might be ascribed in part to this metabolic defect as well as to prolonged portal occlusion

SUMMARY

The lethal postoperative shock associated with transposition of the portal vein and inferior vena cava above the adrenal vein has been shown to be due to adrenal insufficiency This results from hepatic inactivation of adrenal corticoids which are diverted directly into the liver following this operation

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vena cava and anastomosed to the severed portal vein at the hilus of the liver 1 wk following the original side to side portacaval anastomosis Vena caval flow was then diverted to the liver via the graft by the placement of a ligature above the graft anastomosis Both of these procedures avoided even temporary occlusion of portal venous drainage and in none of the animals were there technical difficulties yet all 8 animals expired in unexplained postoperative shock

In the belief that the unexplained postoperative shock might be due to liver damage and to the effect of hepatic anoxia[5,6] attempts were made to perfuse oxygenated blood into the liver during and after the operative procedure In 5 dogs this was attained by anastomoses of the splenic artery into the portal vein close to the hilus of the liver In 2 animals the anastomosis was left in place after the operation while in 3 other animals the splenic artery was ligated shortly after completion of the venous transposition Postoperatively oxygenated blood was infused into the liver via the femoral vein but all of the animals expired within 18 hr of unexplained shock

This experience suggested that a metabolic rather than a technical difficulty was responsible for the shock following portacaval venous transposition since all adrenal venous drainage was diverted directly into the liver following this operation Since adrenal corticoids are known to be inactivated by the liver[7,8] functional hypoadrenal corticoidism might thus be produced

*On this basis the animals were prepared preoperatively with 3 daily in*tramuscular injections of 50 mg of cortisone acetate and maintained on a continuous infusion of 50 mg of Compound F during operation Postoperatively cortisone 50 mg daily was given for from 2 to 8 days Of the 16 animals given such adrenal hormone therapy 4 died postoperatively from known technical causes but the remaining 12 dogs were long term survivals and had an uneventful postoperative course without evidence of hypotension

One animal given Compound F during the operation without preoperative preparation expired in shock shortly after the infusion was discontinued

## DISCUSSION

In the original description of portacaval transposition Child[1] noted the frequent occurrence of postoperative mortality due to shock Such mortality has been confirmed by others[2] but has been materially reduced by temporary clamping of the superior mesenteric artery during the period of venous occlusion and by giving blood transfusions during and after the procedure[1,2] In the operation as described by Child the right adrenal vein is severed in order to obtain more adequate mobility of the vena cava whereas in this study the adrenal veins were left intact and the anastomosis performed proximal to this point In the former technique some of the adrenal drainage might therefore bypass the liver whereas in the latter case all adrenal hormones flow directly into the liver

The thoracoabdominal incision utilized in this study in order to perform a high line of anastomosis undoubtedly is more traumatic to the animal than the subcostal approach employed by Child This might also contribute to the increased mortality of the procedure in an animal already in a borderline state of adrenal insufficiency

*Table 1 Changes in Blood Ammonia in 5–6 Hour Period*

| GROUP | NO OF DOGS USED | FEEDINGS | NO OF OBSERVATIONS | MEAN CHANGE IN BLOOD $NH_3$ LEVEL | | STANDARD ERROR OF DIFFERENCE OF MEANS |
|---|---|---|---|---|---|---|
| 1 | 4 Normal | 1000 cc Blood | 14 | $-4\gamma\%$ | ±30 S D | |
| 2 | 3 Eck Fistula | Milk and Mash | 12 | $+48\gamma\%$ | ±125 S D | |
| 3 | 5 Eck Fistula | 200 cc Blood | 12 | $+165\gamma\%$ | ±376 S D | Group 2 3 114 |
| 4 | 3 Eck Fistula | 400 cc. Blood | 7 | $+193\gamma\%$ | ±147 S D | Group 2-4 66 25 |
| 5 | 2 Eck Fistula | 700 cc Blood | 14 | $+314\gamma\%$ | ±296 S D | Group 2 5 79 5<br>Group 1 5 79 7 |

ml of blood following the afternoon blood ammonia determination A period of 18 hr was then allowed to elapse before the next determination Thus each dog received a total of 1050 ml of blood over a 24 hr period A series of 10 observations was recorded in this phase of the experiment

RESULTS

Table 1 records the results of the blood feeding experiment It indicates that in normal dogs the feeding of 1000 ml of blood produces no real change in the blood ammonia level at the end of a 5 to 6 hr period The change of minus $4\gamma\%$ found in this group represents only an arithmetical mean change and is so slight as to be impossible to measure accurately with present techniques When Eck fistula dogs were fed milk and mash a mean rise of $+48\gamma\%$ resulted at the end of a 5 to 6 hr period This we have interpreted as representing the mean change occurring in Eck fistula dogs 5 to 6 hr after they have consumed a standard diet When the Eck fistula dogs were fed 200 ml of blood a mean rise of $165\gamma\%$ resulted However this rise was not significant when compared with the mean change found after feeding milk and mash using twice the standard error of the difference of the two means as a measure of significance When 400 ml of blood were fed to Eck fistula dogs a rise of $193\gamma\%$ resulted This rise was significant when compared with the mean rise seen following the milk and mash diet When 700 ml of blood were administered to Eck fistula dogs the mean rise of $314\gamma\%$ compared with the rise of $48\gamma\%$ seen following the milk and mash feedings demonstrated most clearly that a significant rise in the blood ammonia could be produced by the digestion of blood in the gastrointestinal tract

Table 2 represents the results of feeding blood to Eck fistula dogs with resultant blood ammonia changes in relation to time When the dogs were fed 700 ml of blood a mean rise of $314\gamma\%$ was found at the end of a 5 to 6 hr period If these same dogs were given an additional 350 ml of blood at the end of the 5 to 6 hr period and a period of 18 hr was allowed to elapse before the next blood ammonia determination it was found that a fall occurred during the 18 hr period almost equalling the rise that occurred in the 5 to 6 hr period earlier in the day Thus the mean change in the blood ammonia level over a 24 hr period during which time a total of 1050 ml had been given was only $31\ 9\gamma\%$ Many of our Eck fistula animals exhibited this characteristic of being able to effect a reduction in the blood ammonia level to baseline or prefeeding level if allowed sufficient time between feedings However there were 2 exceptions to this observation

# DIGESTION OF BLOOD IN THE GENESIS OF HEPATIC COMA*

Harold F. Welch, Joseph C. Pender and John E. Kiley

The observation that hepatic coma in patients with cirrhosis is so often associated with the onset of esophageal bleeding has suggested to us that the bleeding itself must be directly responsible for the initiation of the coma. Previous investigators have shown that a diet rich in protein will, in some cirrhotic patients, evoke the signs and symptoms of hepatic coma.[1] It also has long been known that one can produce meat intoxication in Eck fistula dogs by giving them an excess of animal protein. Thus it is not unreasonable to assume that blood, which contains 175 gm. of protein/L, may, when introduced into the bowel as in a hemorrhage, furnish sufficient nitrogen to cause hepatic coma in patients with severe liver disease. In order to test this hypothesis in dogs with deranged liver function, we prepared a series of Eck fistula dogs and then fed them varying quantities of blood and observed their responses to these feedings and the changes produced in their blood ammonia levels.

## METHOD

Using an end-to-side portocaval shunt, we produced an Eck fistula in 7 dogs and then fed these dogs 200 to 700 ml. of banked outdated human blood through a gastric tube. Blood ammonia determinations were carried out twice daily using the method of Conway, the first in the midmorning, the second in the midafternoon approximately 5 to 6 hr. after the morning value. The dogs were fed shortly after the determination of the morning blood ammonia level. If the feedings were less than 300 ml., the blood was given in 1 feeding; if the amount was greater than 300 ml., the total amount was given in 2 feedings an hour apart. This was done to reduce the incidence of vomiting which was common when larger amounts were given.

A total of 21 observations was recorded. Each represented a feeding with the resultant change in the blood ammonia level. There were 12 observations regarding the changes following the feeding of 200 ml. of blood, 7 following the feeding of 400 ml. of blood, and 11 following the feeding of 700 ml. of blood. As controls, 4 normal dogs were given 1000 ml. of blood in two 500 ml. feedings, with the first feeding given shortly after the morning ammonia determination. Thus, as in the Eck fistula dogs, a period of 5 to 6 hr. elapsed between the 2 determinations. A total of 14 observations were carried out on the control dogs.

Three Eck fistula dogs were fed a diet of milk and mash in order to determine the daily variations in blood ammonia levels occurring in animals on a standard diet. Blood ammonia levels were determined on the same schedule as for the preceding groups of animals. A series of 12 observations was recorded for this group.

In order to assess further the time factor in relation to the feedings and to the changes in the blood ammonia levels, those 2 dogs to whom 700 ml. of blood had been administered in the morning were given an additional 350

*From the Departments of Surgery, Albany Medical College, Albany Hospital and Albany Veterans Administration Hospital, Albany, New York. Aided by a grant from the U. S. Public Health Service.

*Table 3 Clinical Cases*

| | Survived | Died | Died with both continuing hemorrhage and coma | Died with continuing hemorrhage but without coma | Died in coma after hemorrhage stopped | Died from causes other than continuing hemorrhage or coma |
|---|---|---|---|---|---|---|
| 19 Cases Without Elevated Blood Ammonia | 9 | 10 | 1 | 7 | 0 | 2 |
| 22 Cases With Elevated Blood Ammonia | 3 | 19 | 12 | 0 | 0 | 7 |

As a clinical corollary to these experimental observations the progress of 35 consecutive patients with liver disease and alimentary tract hemorrhage was followed in the hospital These cases are analyzed in Table 3

There were 19 patients without elevated blood ammonia levels Two of the 10 deaths in this group were attributed to causes other than coma or hemorrhage while the remaining 8 patients died of exsanguination Only 1 of these patients was comatose at the time of death Of the 22 patients with elevated blood ammonia levels only 3 survived Seven of the 19 deaths in this group were from causes other than hemorrhage or coma Several deaths were related to operative intervention for the purpose of hemostasis All 12 of the remaining patients died with continuing hemorrhage and coma It is especially noteworthy that in this series continued hemorrhage was the commonest cause of death and no patient died in coma once the bleeding was controlled

## CONCLUSIONS

These observations on animals and patients support the hypothesis that the coma often observed following gastrointestinal hemorrhage in severe liver disease is frequently caused by ammonia intoxication This ammonia intoxication results from inability of the diseased liver to metabolize in a normal manner ammonia derived from the protein of the blood lost into the gastrointestinal tract Stopping the hemorrhage is thus an important factor in the prevention of this type of hepatic coma

## REFERENCE

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*Table 2 Blood Ammonia Changes in Relation to Time*

| AMOUNT OF BLOOD FED | NO. OF OBSERVATIONS | TIME ELAPSED AFTER FEEDINGS | MEAN CHANGE IN BLOOD $NH_3N$ LEVELS |
|---|---|---|---|
| 700 ml | 14 | 5-6 Hr<br>A.M.——P.M. | +314γ% |
| 300 ml | 10 | 18-19 Hr<br>P.M.——A.M. | −108γ% |
| 1000 ml | 10 | 24 Hr<br>A.M.——A.M. | +3[illegible]γ% |
| AVERAGE A.M. BLOOD $NH_3N$ BEFORE FEEDING 278γ% (4 Obs.) | | | |
| AVERAGE A.M. BLOOD $NH_3N$ AFTER FEEDING 1000 ml/day 288γ% (10 Obs.) | | | |

The first exception was those Eck fistula dogs who maintained continuously high blood ammonia levels on a low protein diet. These animals appeared chronically ill and if fed protein even in minute amounts would exhibit elevations out of proportion to the response expected in Eck fistula animals. More significant, however, was the observation that in these animals the fall in the blood ammonia level did not equal the rise caused by the feeding but that each feeding usually would cause a progressive rise in the baseline blood ammonia level. These Eck fistula dogs rapidly developed signs and symptoms of meat intoxication, while those animals in whom the fall in the blood ammonia equalled or nearly equalled the rise, leaving the baseline virtually unchanged at the end of a 24 hr. period, usually did not develop signs and symptoms of meat intoxication even though the blood ammonia level often rose to 4 or 5 times the prefeeding baseline level at some time during the 24 hr. period.

The second exception, apparent from earlier meat feeding experiences, was that by spacing the feedings over short periods of time we were able to overwhelm the animals with excessive amounts of protein. In this manner it was possible to sustain a fairly high blood ammonia level in many of the Eck fistula dogs.

Two Eck fistula dogs died as the result of digestion of blood in the bowel. Both animals had markedly elevated blood ammonia levels and exhibited the characteristic signs and symptoms of meat intoxication. The first animal which had a chronically elevated blood ammonia was fed 1200 ml. of blood in 6 feedings over a 36 hr. period with the deliberate intention of producing coma. The dog died in coma 14 hr. after the first feeding with a blood ammonia level of 840γ%. The second animal was an Eck fistula dog who had not been fed blood. Previously in good health, this dog approximately 34 hr. before its death developed progressive signs and symptoms of meat intoxication and gradually lapsed into coma and died in coma with a blood ammonia level of 780γ%. The dog received transfusions and fluid therapy for 12 hr. preceding its death and did not appear to be at any time in a state of shock. Autopsy revealed a patent portacaval shunt with the stomach and small bowel filled with blood. The source of the hemorrhage was numerous small ulcerations of the gastric mucosa. No other factors contributing to the animal's death could be found.

as described by Harper *et al*[7] Samples of blood were drawn immediately be fore the infusion of glycine and at 30 min intervals thereafter, for a 2 hr period Heparin was used as the anticoagulant The samples of blood were analyzed for ammonia nitrogen by the microdiffusion method of Conway[8] and for urea nitrogen by the method of Van Slyke and Kugel[9]

The effect on blood ammonia of arginine or of monosodium glutamate was evaluated by two types of experimental procedures

1 Infusions of monosodium glutamate or of arginine were administered at a rate of 1 75 mg amino N/kg /min concurrently with glycine at a rate of 3 5 mg amino N/kg /min and the levels of ammonia in the blood were compared with those obtained during the infusions of glycine alone

2 Concentrations of ammonia sufficient to produce toxicity were allowed to accumulate in the blood as a result of continuous infusions of glycine at 3 5 mg amino N/kg /min for 1 hr at which time the glycine infusion was continued at the same rate but 25 gm of arginine or 50 gm of monosodium glutamate were added and the mixture was administered for an additional hour

## RESULTS

The infusion of glycine alone produced a marked rise in blood ammonia but when arginine was administered together with glycine this rise was pre vented In fact at the termination of the injection the reduction of blood ammonia over that of the control was tenfold Under the same experimental conditions monosodium glutamate was able to effect only a slight decrease in blood ammonia A comparison of the concentrations of ammonia in the blood after infusion of glycine alone glycine with monosodium glutamate and glycine with arginine are shown in Figure 1a

The changes in the urea nitrogen of the blood that followed injection of arginine when compared to those after infusion of monosodium glutamate suggested that the superior effect of arginine in preventing a rise in am monia was due to increased production of urea (Fig 1b)

The levels of ammonia and urea in the blood that occurred after the de layed injection of arginine or of monosodium glutamate are shown in Fig ures 1c and 1d In these experiments the ability of arginine to bring about a reduction in the blood ammonia was clearly shown Under the same condi tions however monosodium glutamate proved ineffective although given at twice the dose of arginine

The ability of arginine to decrease significantly or to prevent a rise in blood ammonia levels under experimental conditions suggested a trial of this agent in clinical disease states associated with elevated blood ammonia levels The effect of intravenous administration of arginine was therefore studied in 20 patients with elevated blood ammonia levels and varying de grees of encephalopathy In every instance a reduction in the levels of am monia in the blood occurred following arginine therapy The most satisfac tory results were obtained in cases with acute ammonia intoxication secon dary either to massive hemorrhage or to ammonium chloride ingestion In those patients with chronically elevated blood ammonia the response was somewhat more delayed However in each instance improvement in the mental status of the patient accompanied the reduction of blood ammonia levels In over half the cases the patients were initially in deep coma how

# A COMPARISON OF ARGININE AND L-GLUTAMIC ACID IN THE TREATMENT OF AMMONIA TOXICITY*

JOHN S NAJARIAN AND HAROLD A HARPER

The toxic effects that follow the experimental elevation of ammonia in the blood in dogs have been described by Koprowski and Uninski[1] Disturbances of the central nervous system similar to those which occurred in the experimental animal after the administration of ammonium salts have also been reported in patients with elevated levels of ammonia in the blood [3] These findings have stimulated increased interest in the possible role of ammonia in clinical disease[4]

The problem is important to the surgeon because two of the most common clinical syndromes associated with elevated levels of ammonia in the blood may occur either in patients who have had vascular shunting procedures performed to ameliorate portal hypertension or in those in whom chronic liver disease is associated with gastrointestinal hemorrhage

The treatment of patients with elevated blood ammonia levels requires the use of measures to control the amount of ammonia derived from the gastrointestinal tract as well as efforts to reduce its concentration in the blood The production of ammonia in the gastrointestinal tract can be controlled by (1) limitation of the intake of protein (2) control of gastrointestinal bleeding and prompt removal by catharsis or lavage of blood which may have accumulated from hemorrhage and (3) oral administration of antibiotics in an effort to diminish the activity of the intestinal bacteria which produce ammonia from nitrogenous substrates The administration of ammonium salts or of resins containing ammonia must also be avoided in these patients

Reduction of the concentration of ammonia in the blood may be accomplished by enhancement of the reactions by which it is normally metabolized and removed from the body These reactions include its excretion as ammonium salts or as urea or its detoxification by transamination The rationale for the glutamic acid therapy of ammonia intoxication proposed by Walshe[5] and McDermott[6] is based on removal of ammonia by amidation of the glutamic acid to form glutamine The formation of urea however is quantitatively the most important method by which ammonia is removed from the body Experiments were therefore undertaken to lower blood ammonia levels by attempting to increase urea production

Arginine is the amino acid which is the immediate precursor of urea in the Krebs Henseleit cycle The effect of arginine as well as that of glutamic acid on the blood ammonia was therefore studied under the same experimental conditions in order to evaluate the relative efficiency of urea production as compared to transamination for detoxification of ammonia

## METHOD

Ammonia intoxication was induced in adult mongrel dogs by rapid intravenous administration of glycine at a rate of 3 5 mg of amino N/kg /min

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*From the Surgical Research Laboratories of the University of California School of Medicine San Francisco Supported by the Christine Breon Fund for Medical Research

ever with 1 exception all became alert and had a clear sensorium within 24 to 48 hr following arginine therapy


## SUMMARY

1 The effects on the blood ammonia of simultaneous or delayed administration of either L arginine hydrochloride or L monosodium glutamate were studied in dogs

2 Arginine was much more effective than monosodium glutamate either in preventing a rise or in reducing the levels of ammonia in the blood

3 When arginine was given the rise in blood urea that accompanied the marked fall in blood ammonia indicated that this amino acid exerted its effect on the blood ammonia by an influence on the production of urea

4 Intravenously administered arginine was also found to be effective in lowering elevated levels of ammonia in the blood of patients with clinical manifestations of encephalopathy of varying degrees



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# MORTALITY FACTORS IN EXPERIMENTAL HEMORRHAGIC PANCREATITIS*

Irvin H Sokolic and Alex W Ulin

The factors responsible for the high mortality in acute hemorrhagic pancreatitis in man have been variously ascribed to 1) infection 2) a specific circulating toxic material of pancreatic enzyme origin 3) release of non specific toxic materials due to marked tissue necrosis 4) disturbed fluid and electrolyte balance and 5) reduced effective circulating blood and plasma

*From Dept of Surgery Hahnemann Medical School and Hospital and Albert Einstein Medical Center Southern Division Philadelphia Pa With the technical assistance of Dr John Kachmar Mr William Chambres and Miss Marcelle Rosenberg

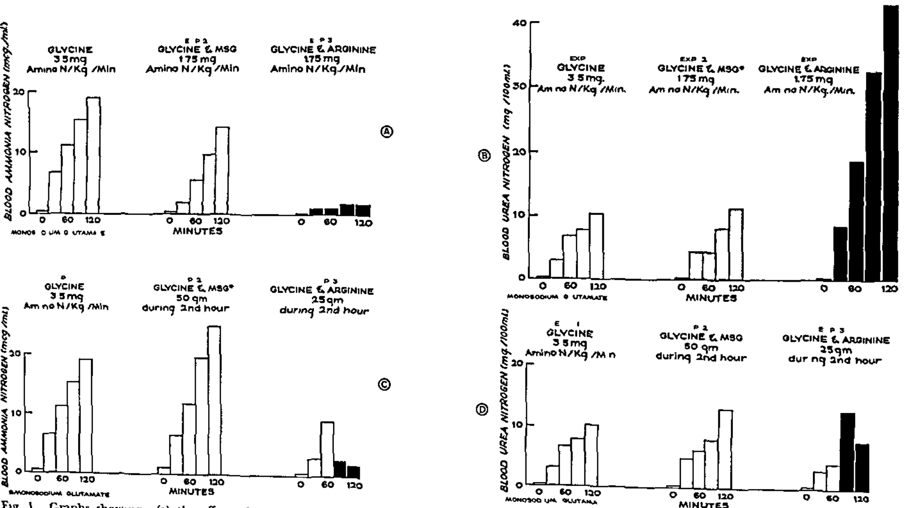



Fig 1 Graphs showing (a) the effect of intravenous infusion of various amino acid mixtures on the levels of ammonia in the blood (b) the effect of intravenous infusion of various amino acid mixtures on the levels of urea nitrogen in the blood (c) the effect of monosodium glutamate and arginine on elevated levels of ammonia in the blood Glycine was given for 2 hr in Experiment 1 In Experiment 2 glycine was given for the first hour and a mixture of glycine and monosodium glutamate was given during the second hour In Experiment 3 arginine was added t the glyc ne during the second hour (d) Blood urea nitrogen after admin tration of mo o o lium gluta nate o argini e n the presence of le te l 11 1 n monia levels The experiment was carr ed out a in licate l in (c)

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SUMMARY

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The factors responsible for the high mortality in acute hemorrhagic pancreatitis in man have been variously ascribed to 1) infection 2) a specific circulating toxic material of pancreatic enzyme origin 3) release of non specific toxic materials due to marked tissue necrosis 4) disturbed fluid and electrolyte balance and 5) reduced effective circulating blood and plasma

*From Dept of Surgery Hahnemann Medical School and Hospital and Albert Einstein Medical Center Southern Division Philadelphia Pa With the technical assistance of Dr John Kachmar Mr William Chambres and Miss Marcelle Rosenberg

volume Each of these factors was investigated in turn by the techniques to be described below

As a control for evaluation of further studies acute hemorrhagic pancreatitis with necrosis was produced in 6 dogs by the method of Archibald[1] namely by injecting 10 cc of gallbladder bile into the main duct of the pancreas using an injection forcible enough to produce duct rupturing Rupture of the ducts was attested to by the fact that the bile tended to distribute itself throughout the pancreatic parenchyma and in the interlobular spaces This was further confirmed by microscopic study Intravenous nembutal and pentothal anesthesia was employed and postoperative care was limited to the supportive use of parenteral fluids when indicated This routine was also followed in all cases to be described below Serial serum amylase and lipase studies were also carried out As has been demonstrated in the past amylase values normally approximately 2 000 units in the dog rose to values between 12 000 and 32 000 Somogyi units The typical gross lesions of necrotizing pancreatitis were seen on autopsy examination of the animals at the time of death Bacteriologic study showed the Welch bacillus to be the most common pathogen None of the 6 animals survived more than 72 hours

The role of infection was then investigated by repeating and verifying the work of Fine[2, 3] and his group Pancreatitis was produced in 3 animals by the methods described above Two dogs were treated with intramuscular penicillin and streptomycin and 1 with intramuscular oxytetracycline Antibiotics were given over a 4 day period In each instance amylase values over 8 000 units were obtained but the animals survived Autopsy examination after sacrifice 7 to 10 days after the induction of pancreatitis showed an edematous firm pancreas Hemorrhage into the gland and into the peritoneal cavity was not marked as in the previous preparations These results verified the reports of previous investigators and were pursued no further Thus it appeared that the prevention of infection was of paramount importance in reducing the mortality of bile injection pancreatitis

The escape of specific circulating toxic enzymes with their alleged devastating effects on the body *economy has long been thought to be* a major factor in the poor prognosis of pancreatic necrosis Investigation of this problem was approached in the following manner It has been shown by others[4, 5] that the administration of dl ethionine causes marked histologic alterations of the pancreas These are manifested during the first 5 days by loss of basophilia vacuolization of the cytoplasm and apparent decrease in the amount of zymogen granules of the cells of the acini Also during this time there is a steady fall in the level of peripheral serum enzymes and some evidence to indicate a fall in enzyme content of the pancreatic secretions This has been interpreted to indicate that enzyme production of the pancreas is markedly altered and inhibited by the administration of ethionine If these conclusions are correct then there is afforded us a powerful tool for the study of bile injection pancreatitis namely a pancreas partially devoid of enzyme content Accordingly 27 dogs were given ethionine in doses varying between 25 and 150 mg /kg for a period of 1 to 5 days A typical protocol is reproduced below (See Fig 1 )

In only 2 instances did the animal pretreated with dl ethionine survive the pancreatic insult Thus the mortality of hemorrhagic pancreatitis is not significantly altered by modifying the enzyme content of the pancreas This

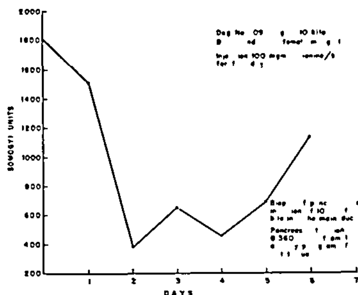



Figure 1

would suggest that the release of pancreatic enzymes is not an important factor in contributing to the high mortality of pancreatitis

The evaluation of the release of non specific toxins from the necrotic tissues was next undertaken With a dog under nembutal anesthesia 10 cc of bile was injected into the main pancreatic duct producing the usual marked edema of the pancreas The entire pancreas was then excised placed in a Waring blender and homogenized To the homogenate was added 100 000 units of penicillin and 0 5 gm of streptomycin The entire tissue mash was then injected into the peritoneal cavity of a second animal through a trocar incision All 3 animals which were injected did well clinically in the immediate postoperative period Amylase values of 5 000 to 9 000 units were recorded yet the animals were alert ate and walked about with none of the stigmata of abdominal catastrophe This indicated to us that the release of nonspecific breakdown products was probably not of importance and this approach was abandoned

The evidence gathered to this point then seemed to indicate that there was nothing inherent within the acutely inflamed pancreas which was in itself lethal The problem of hemorrhagic pancreatitis appears to have evolved itself into an appraisal of the general systemic effects produced by the localized pancreatic disease To test the effect of localized pancreatic disease in parts of the body other than the peritoneal cavity the following technique was devised A series of dogs was submitted to a 2 stage procedure wherein during the first stage the pancreas was split into 2 parts in the manner shown in the following diagram (See Fig 2)

In control animals the distal pancreas was left within the peritoneal cavity and in test animals it was planted into a subcutaneous pouch Seventeen animals survived the procedure In 6 control animals hemorrhagic pancreatitis was then produced by bile injection at a second operation 2 to 3 wks after the original procedure Five of the animals died with postmortem evidence of hemorrhagic pancreatitis and peritonitis

In the 11 test animals the proximal half of the pancreas was excised during the first stage in 8 dogs and allowed to remain in place in 3 dogs Hemorrhagic pancreatitis was then produced in the extraperitoneal pancreas by the

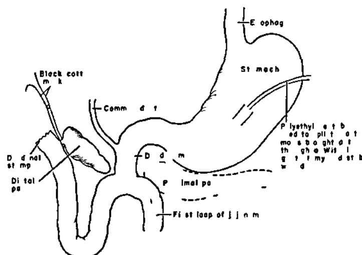



Figure 2

injection of 10 cc of bile (collected and frozen during the first stage) into the main pancreatic duct Two of the animals died in the immediate post operative period However the remaining 9 animals did well clinically The dogs were taking water on the first postoperative day and by the second day were taking food and were quite active Examination on the fourth or fifth postoperative day under anesthesia showed the presence of a hemorrhagic pancreas in the extraperitoneal space associated with 1 to 3 oz of bloody fluid and scattered areas of fat necrosis in the subcutaneous pouch The peritoneum itself showed no evidence of inflammation In the 3 instances where the proximal pancreas had been left intact within the abdomen there was no gross or microscopic evidence of pancreatitis within the intraperi toneal gland

In the interim between procedures when the pancreas was implanted sub cutaneously a moderate amount of scar had formed about the pancreas This was left essentially undisturbed by employing a long black marker thread to lead us to the main duct Thus in this test preparation 2 altera tions were apparent both of which led to survival of the animals First peri tonitis was not produced and second swelling of the pancreas was not permitted because of the encapsulating scar tissue This leads us to believe that the lethal factors causing the high mortality of experimental pancrea titis are (1) peritonitis and (2) marked alterations in blood and plasma volume resulting from hemorrhage into the pancreas and peritoneal cavity with the attendant plasma loss associated with infection

In conclusion then our experiments would indicate that the principal factors causing death in experimental acute pancreatitis are not associated with increased quantities of circulating enzymes or their breakdown prod ucts but are due primarily to peritonitis and shock from infection and blood loss

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# THE ROLE OF PROPYLTHIOURACIL IN PANCREATITIS*

L. Corsan Reid Thomas W Challis Robert L Pauletti
and J William Hinton

Recently it has been shown[1, 2] that thiourea and its analogues owe their antithyroid action in part at least to their antioxidant property in all organs and tissues In other words they interfere with the utilization of oxygen in all organs and tissues As a consequence there is a decrease in the amount of energy released less product is formed and in the case of the thyroid less thyroxine is produced In addition the restriction in the amount of oxygen utilized by all other organs and tissues results in a marked lowering of the basal metabolic rate Propylthiouracil has been a particularly active compound in bringing about these changes

Starr stated at a meeting of the Pan Pacific Surgical Association in Honolulu in October 1951 that propylthiouracil was a valuable therapeutic agent in acute pancreatitis No comments were made about its mechanism of action As a result of this statement and our experience with propylthiouracil the following working hypothesis was evolved which was susceptible of being tested experimentally The results of this investigation constitute the basis of this report

Propylthiouracil inhibits oxygen utilization in the pancreas less energy is released by oxidative cell energy mechanisms less work is done less products are produced and the organ is placed at rest when under severe stress This decreased synthetic activity should permit a larger proportion of the energy released in the organ to be available for the maintenance of the morphological integrity of the cells of the pancreas This is of the utmost importance in preventing the escape of a flood of powerful proteolytic enzymes which bring about almost instantly destructive and irreversible alterations in cell organization and organ structure These particular intracellular enzymes occur only in the pancreas This makes the preservation of the cell structure of the pancreas vastly more important than that of any other organ

In addition the effect of propylthiouracil in lowering the metabolic tempo of all the other organs should serve to decrease further the demands on the pancreas

## METHOD

1 The hypothesis was tested by measuring the effects of propylthiouracil upon the oxygen uptake of slices mince homogenates and mitochondria

*From the Department of Surgery New York University Post Graduate Medical School New York Supported by the Ruth H Kress Grant to the John F Erdmann Surgical Research Laboratory

from the pancreas and comparing these findings with similar preparations under the same conditions with other organs such as liver and kidney by the usual manometric techniques.

2. The average serum amylase levels of normal dogs were established in a large number of animals and compared to animals receiving propylthiouracil.

3. Dogs receiving ACTH were treated with propylthiouracil.

RESULTS

Tissue slices and tissue mince from the pancreas showed moderate oxygen uptake. Homogenates and mitochondria on the other hand showed practically no oxygen consumed under standard conditions. On addition of methylene blue acceptable readings were obtained but these were significantly lower than those found in liver or kidney. On addition of propylthiouracil in increasing quantities there was a progressive linear decrease in oxygen uptake. This was true of all homogenates but was less obvious in the case of the pancreas than other organs.

*Table 1*

| | | MICROMOLS PER ML. OF HOMOGENATE | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ORGAN | CONTROL | 2.4 | 4.8 | 7.2 | 9.6 | 12.0 | 14.4 | 16.8 |
| KIDNEY | 1236 | 1072 | 936 | 714 | 571 | 457 | 350 | 292 |
| | 1132 | 1065 | 892 | 672 | 564 | 432 | 347 | 273 |
| LIVER | 975 | 947 | 884 | 801 | 729 | 602 | 503 | 426 |
| | 974 | | 865 | 793 | 724 | 586 | 476 | 380 |
| PANCREAS | 375 | 366 | 370 | 311 | – | 280 | 227 | 177 |
| | 373 | 352 | 332 | 267 | — | 276 | 226 | 161 |

In the case of the mitochondrial preparations all were exquisitely sensitive to propylthiouracil and in the case of the pancreas this was particularly so (Fig. 1).

Eighteen dogs received 300 mg. propylthiouracil/day for an average of 2 wks. All but 3 showed a marked decrease in the serum amylase level and in 2 of the 3 failures a significant decrease occurred on increasing the dose of propylthiouracil.

Propylthiouracil 75 mg./day was given to 5 dogs on ACTH. The results were equivocal.

DISCUSSION

The oxygen uptake by slices and mince can be satisfactorily explained on the basis that cell organization is relatively intact, energy release mechanisms are operating and oxygen is consumed.

The failure of homogenates and mitochondria to utilize oxygen appears to depend upon the almost instantaneous disorganization of the cell machinery as a result of the rupture of the cell membrane. The addition of methylene blue to the Warburg vessels greatly improved the oxygen utilization in homogenates and mitochondria which was not true under similar conditions in either liver or kidney.

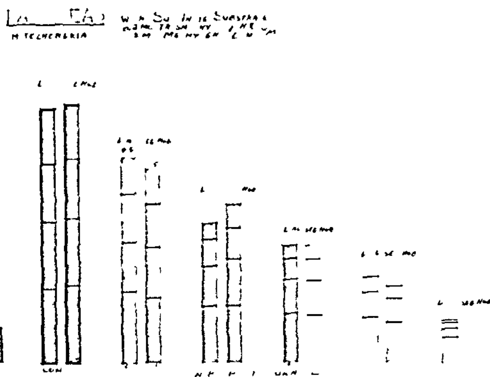


Fig 1

The inhibition by propylthiouracil of oxygen uptake in pancreatic homogenates was not as marked as in other organs and this may well be due to the results of the action of degradative enzymes In the case of the mitochondria less structural disorganization has occurred and they are as sensitive as those from other organs to smaller dosages of the drug

PANCREAS HEART KIDNEY LIVER

Fig 2

When one recalls that 85 per cent of the energy released by the cell is an oxidative process which is exclusively localized in the mitochondria these effects of propylthiouracil upon pancreatic mitochondria take on considerable significance and would appear to have therapeutic possibilities

The rapid and powerful proteolytic activity of the intracellular pancreatic enzymes is clearly shown by the almost complete inhibition of oxygen uptake when one adds pancreas homogenates to liver or kidney homogenates These proteolytic pancreatic enzymes are equally destructive to liver and kidney as well as pancreas (Fig 2) In mixing together any other 2 organ homogenates no such changes are seen

In the 18 dogs receiving 300 mg propylthiouracil/day the lowering of the serum amylase level indicates an inhibition of protein synthesis regardless of the source of serum amylase

Previous work from this laboratory brings rather convincing evidence that the release of ACTH plays a dominant role in the elevation of the serum amylase and the serum amylase level is appreciable even in pancreatectomized dogs with total removal of the salivary glands

On giving 75 mg propylthiouracil/day to 5 dogs on ACTH injections the lack of any clear cut findings may be due to the low dosage employed

SUMMARY

1 Propylthiouracil inhibits oxygen uptake with the result that there is a reduction in the energy supply available to the organ as shown in its action on homogenates and mitochondria from the pancreas

2 Propylthiouracil lowers the serum amylase levels in normal dogs

3 Present experience indicates that propylthiouracil will modify the elevation of serum amylase that results from the release of ACTH under stress

4 These experimental findings suggest that propylthiouracil reduces the tempo of pancreatic metabolism by its direct anti-oxidant action on the pancreas itself and indirectly by a similar action on all other body organs and tissues This serves to place the organ at rest when under stress This should be a therapeutically useful procedure in pancreatitis by making more energy available for maintaining the anatomical and cellular integrity of the organ at the price of lowered functional activity

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# DIAGNOSTIC VALUE OF URINARY LIPASE*

Allan D Callow Martin M Nothman and Joseph H Pratt†

Because studies on fat splitting urinary enzymes are rare and results are contradictory this investigation has attempted to confirm the existence of such an enzyme in urine to demonstrate that its chief source is the pancreas and to consider its usefulness as a diagnostic aid

## METHOD

Utilizing a method previously described[1] a fat splitting enzyme has been regularly found in the urine of dogs[2] A urine-olive oil emulsion mixture was incubated for 24 hr for the titration of liberated fatty acids to a pink end point with phenolphthalein and N/20 sodium hydroxide The amount of sodium hydroxide required for such titration usually 0 1 cc to 0 75 cc has been expressed as units of the enzyme One hundred and fifty samples of urine from normal dogs were tested and values were found to range from 0 15 to 1 0 units Injection of secretin or methacholine (Mecholyl) increased the amount of enzyme in the urine Removal of the pancreas resulted in immediate disappearance of the enzyme in the urine Ligation of the pancreatic ducts produced a marked increase in the concentration of the urine whereas ligation of the pancreatic ducts followed by pancreatectomy produced a rapid disappearance in the previously high urinary enzyme values It has been concluded that the fat splitting enzyme is of pancreatic origin

A fat-splitting enzyme has also been detected regularly in the urine of man[3] Utilizing the same method of titration of liberated fatty acids 500 samples of urine from healthy individuals yielded values which ranged from 0 1 to 0 75 units Values of 1 0 units or more were considered significantly elevated Urine samples collected at 2 hr intervals for 24 and 48 hr from male patients free of gastrointestinal signs and symptoms and who had received secretin and mecholyl by injection yielded characteristic values for the amounts of fat-splitting enzyme Values regularly rose 2 hr after secretin administration reached a maximum in 6 to 8 hr and returned to normal in 24 hr The normal peak response was 1 2 units No significant difference occurred in the values of urinary lipase in the same individual during the same day or on consecutive days Values ranged between 0 2 and 0 45 units in 1 individual whose urine was tested 3 times daily for 7 days and in another individual varied from 0 35 to 0 55 units There is apparently no change in the concentration of the urinary lipase as a result of fasting or ingestion of food In 25 cases the total 24 hr volume of urine was tested and revealed no significant variation on a day to day basis of urinary lipase In 12 cases of pregnancy the volume of urinary lipase ranged again from 0 1 to 0 75 units The patients tested were in their 3rd to 7th months of gestation

Thirteen cases of carcinoma of the pancreas established by subsequent surgical exploration were found to yield normal values for urinary lipase when such patients did not have secretin or mecholyl stimulation Injection

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When one recalls that 85 per cent of the energy released by the cell is an oxidative process which is exclusively localized in the mitochondria these effects of propylthiouracil upon pancreatic mitochondria take on considerable significance and would appear to have therapeutic possibilities

The rapid and powerful proteolytic activity of the intracellular pancreatic enzymes is clearly shown by the almost complete inhibition of oxygen uptake when one adds pancreas homogenates to liver or kidney homogenates These proteolytic pancreatic enzymes are equally destructive to liver and kidney as well as pancreas (Fig 2) In mixing together any other 2 organ homogenates no such changes are seen

In the 18 dogs receiving 300 mg propylthiouracil/day the lowering of the serum amylase level indicates an inhibition of protein synthesis regardless of the source of serum amylase

Previous work from this laboratory brings rather convincing evidence that the release of ACTH plays a dominant role in the elevation of the serum amylase and the serum amylase level is appreciable even in pancreatectomized dogs with total removal of the salivary glands

On giving 75 mg propylthiouracil/day to 5 dogs on ACTH injections the lack of any clear cut findings may be due to the low dosage employed

SUMMARY

1 Propylthiouracil inhibits oxygen uptake with the result that there is a reduction in the energy supply available to the organ as shown in its action on homogenates and mitochondria from the pancreas

2 Propylthiouracil lowers the serum amylase levels in normal dogs

3 Present experience indicates that propylthiouracil will modify the elevation of serum amylase that results from the release of ACTH under stress

4 These experimental findings suggest that propylthiouracil reduces the tempo of pancreatic metabolism by its direct anti-oxidant action on the pancreas itself and indirectly by a similar action on all other body organs and tissues This serves to place the organ at rest when under stress This should be a therapeutically useful procedure in pancreatitis by making more energy available for maintaining the anatomical and cellular integrity of the organ at the price of lowered functional activity

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# *Tumor Problems and Transplantation*

## LOCALIZATION OF $P^{32}$ AS A DIAGNOSTIC AID IN GASTROINTESTINAL NEOPLASMS*

DONALD B. SHAHON, J. BRADLEY AUST AND HARLAN D. ROOT

Neoplastic tissues, because of their more rapid growth rate, have been found to take up a measurably higher percentage of radioactive phosphorus than normal tissues.[1] Incorporation of phosphorus in the nucleoproteins of neoplastic tissue is several times greater than in normal tissues which have a slower rate of mitoses.[2,3] This property of radioactive phosphorus ($P^{32}$) has had clinical application in the diagnosis of neoplasms of the breast,[4] brain[5,6] and lung.[7] We have applied this technique to the study of patients clinically suspected of gastrointestinal cancer who had undergone surgical resections of their involved organs.

### METHOD

Radioactive phosphorus ($P^{32}$) has a half life of 14.3 days. Its maximum energy of beta radiation is 1.71 million electron volts (Mev.), an average of 0.695 Mev., with a maximum tissue penetration of 0.8 cm. This is entirely beta radiation since there is no gamma radiation from the isotope.

The radioactive phosphorus ($P^{32}$) was obtained from the Oak Ridge National Laboratories. Approximately 0.5 mc. of $P^{32}$ was injected intravenously 4 to 48 hr. prior to surgery in each of the 78 patients with suspected malignancies of the esophagus, stomach, colon or rectum. Following removal of the diseased organ, each specimen was surveyed employing a mica window Geiger-Muller tube, 2 gm./cm.$^2$ in thickness, obtained from the Anton Electronic Laboratories, New York City. A lead shield was placed around the window with an aperture of 0.8 cm. in diameter projecting 0.4 cm. beyond the window. The Geiger-Muller tube was attached to a Berkeley Decimal Geiger-Muller Scaler, model 1000 B. Multiple 1 min. counts of the cancerous area and the adjacent normal mucosa were obtained for differential study. Background counts were obtained in each instance. The cancer-normal ratio of 1.3:1 or greater radioactivity as described by Nakayama[8] was arbitrarily considered as being diagnostic of malignancy. Histologic verification of the diagnosis in the malignant and normal areas studied were obtained in all instances.

### RESULTS

Seventy-eight patients were studied employing the method described above and the results are summarized in Table 1. The differential counts of

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*From the Department of Surgery, University of Minnesota Medical School, Minneapolis 14, Minn. Supported by Malignant Disease Research Fund, American Cancer Society Institutional Research Grant #IOF, Jay and Rose Phillips Fund, Arthur and Stella Sanford Fund.

of secretin in these patients however resulted in a decrease in the concen tration and in some cases the complete disappearance of the enzyme There seemed to be no difference to response to secretin whether the tumor was located in the head of the pancreas (6 cases) or in the body or tail of the pancreas (7 cases) An explanation for this possible exhaustion phenomenon is not available and further studies are in progress

A fat splitting enzyme apparently identical with pancreatic lipase of the dog has been regularly detected in the urine of man Variations in the amount of urinary fat splitting enzymes by the same individual are very slight although variations in amount between different individuals do occur within limitations Changes in the rate of urinary excretion of a fat splitting enzyme apparently occur in the diseased pancreas Further studies are re quired for the interpretation of these changes

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*Table 1 Results of $P^{32}$ Uptake in Resected Gastrointestinal Lesions*

| ORGAN | DIAGNOSIS | NO OF PATIENTS | POSITIVE DIAGNOSIS* | PER CENT CORRECT |
|---|---|---|---|---|
| Esophagus | Epidermoid carcinoma | 6 | 6 | 100 0 |
| Stomach | Adenocarcinoma (polypoid ulcerative and superficial spreading) | 32 | 32 | 100 0 |
| | Linitis plastica | 5 | 0 | 0 0 |
| | Benign gastric ulcers | 7 | 3 | 42 9 |
| Colon | Adenocarcinoma | 15 | 15 | 100 0 |
| | Benign polyps | 1 | 1 | 100 0 |
| | Benign ulcers | 1 | 1 | 100 0 |
| Rectum | Adenocarcinoma | 11 | 10 | 90 9 |

*Counts higher than 1 3 1 equalled positive diagnosis of cancer

cancer normal in all cases with histologically proven malignancy ranged from 1 2 1 to 13 5 1 The counts in all patients with cancer of the esophagus ranged between 1 6 1 and 4 5 1

Cancer normal counts in 37 patients with cancer of the stomach ranged from 1 3 1 to 13 5 1 in 32 specimens all of which demonstrated grossly and microscopically, polypoid ulcerating or superficial spreading carcinomas Approximately 60 per cent of this group revealed differentials of 2 0 1 or higher One patient a 65 year-old female underwent exploratory laparotomy for symptoms of persistent epigastric distress and achlorhydria Roentgenologic examination of the stomach had failed to reveal any lesion At surgery a very slight thickening in the antrum was the only abnormality noted A subtotal gastrectomy was performed Gross examination of the resected stomach by the pathologist failed to reveal any lesion The specimen was then surveyed by Geiger Muller tube which resulted in localizing an area on the anterior wall of the antrum which counted 1 5 times the normal Microscopic sections taken through this area demonstrated a minute and very early superficial spreading carcinoma of the stomach

Multiple benign gastric polyps were noted in 1 patient with an associated carcinoma of the stomach Differential counts of 1 3 to 1 4 1 were obtained in the nonmalignant polyps with no differentiation of the polyps from the carcinoma

Diagnostic counts were unobtainable in 5 patients with linitis plastica Diffuse submucosal involvement of the entire resected specimen without ulceration was noted microscopically in all 5 specimens Due to the absence of any normal stomach for differentiation from the cancer diagnostic counts were not obtainable

Four of the 7 specimens with histologically proven benign gastric ulcers showed differential counts ranging from 1 4 to 2 1 1 All demonstrated acute inflammatory changes or fibroblastic activity in the involved area Since phosphorus is taken up to a greater degree by rapidly growing tissues the increased cellular activity noted in these lesions characterized by inflammatory and fibroblastic activity could account for the high uptake in these 4 instances

Differential counts in 16 colon specimens with microscopic verification of carcinoma ranged from 1.3:1 in 1 patient to 3.2:1 in the remaining 15. Cancer:normal ratios of 2.0:1 or higher were noted in over 60 per cent of this group. Two colon specimens with nonmalignant lesions (one—multiple benign polyps, and the other—superficial nonmalignant ulcerations) had count ratios less than 1.3:1. In 1 instance of carcinoma of the descending colon an associated villous papilloma in the upper sigmoid was encountered. Differential counts of this lesion were similar to those in the cancer. Microscopic examination of the papilloma failed to reveal any malignant change.

An interesting differential count was obtained in 1 patient with multiple polyps of the sigmoid colon. One polyp had been previously biopsied and diagnosed microscopically as adenocarcinoma. Examination of the resected specimen revealed several polyps ranging from 0.3 cm. to 2.0 cm. in diameter. Differential counts of several of the polyps revealed 2 to record high ratios of 2.9:1 with the remaining polyps failing to demonstrate any differentiation. These 2 polyps both proved to be malignant while those with no differentiation failed to demonstrate microscopically any malignant changes.

Cancer:normal ratios of the 11 patients with cancer of the rectum ranged from 1.2:1 in 1 patient to 3.3:1 in the remaining 10 patients. Differential counts of 2.0:1 or greater were observed in 5. Excluding those patients with linitis plastica, the only specimen with proven malignancy that failed to demonstrate a cancer:normal ratio of 1.3:1 or greater was encountered in this group. Gross observation of this specimen revealed an ulceration in the distal rectum approximately 5 cm. by 3 cm. in diameter. Microscopic examination revealed the tumor cells to be well differentiated with no marked increase in mitoses.

Radioautographs of the resected specimens were obtained. Figure 1 demonstrates a positive radioautograph diagnostic of a gastric cancer. The dark



Fig. 1. Radioautograph of carcinoma of stomach.

ened area shows the radioactive phosphorus uptake in the region of the carcinoma

The use of minute Geiger Muller tubes passed through the oral cavity into the esophagus and stomach for surveying these organs *in vivo*, in order to differentiate carcinoma from normal tissue has been initiated This is a report in progress and at present there are insufficient studies to warrant any definite conclusions

## DISCUSSION

The results obtained from this study have confirmed the reports of other investigators malignant neoplastic tissue incorporates phosphorus in greater quantity than normal tissue This was noted in all cancerous specimens studied excluding 1 specimen with a carcinoma of the rectum The 5 cases of linitis plastica failed to demonstrate any normal tissue for differentiation from the cancer False positive determinations were noted in 6 instances 4 with benign gastric ulcers 1 with villous papilloma of the rectum and 1 with multiple gastric polyps These findings can be ascribed to the increased cellular activity as compared to the normal tissues in these organs The use of $P^{32}$ as an additional means of localization and diagnosis in gastrointestinal neoplasms appears warranted Surveying of the more accessible organs *in vivo* such as the esophagus, stomach and rectum by means of minute Geiger Muller tubes passed into these viscera would be of immeasurable value for localization and diagnosis of neoplasms Differential counting at surgical exploration could enhance the performance of a more curative operation by localization of lymph node spread

## SUMMARY

1 Seventy-eight surgically resected specimens of patients with suspected gastrointestinal malignancies were studied employing $P^{32}$ administered intravenously A cancer normal ratio of 1 3 1 was the criteria accepted for a diagnosis of malignancy

2 The method of administration and counting are described

3 Excluding 5 cases of linitis plastica in which differentiation between cancer and normal tissue was not possible due to the absence of adjacent normal stomach adequate localization of radioactive phosphorus in the neoplasm failed to occur in only 1 instance False positive counts suggesting a diagnosis of cancer were noted in 4 of 9 patients with benign gastrointestinal lesions

4 The application of this technique as an aid in the diagnosis localization and surgical treatment of gastrointestinal neoplasm is discussed

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# FURTHER OBSERVATIONS ON THE LYMPHATIC PICK UP OF RADIOACTIVE SILVER COATED GOLD COLLOID ADMINISTERED INTRATHORACICALLY TO DOGS*

R. A Matuska P F Hahn R I Carlson S H Auerbach and G R Meneely

In a previous publication[2] this laboratory reported that radioactive silver coated gold colloid injected into the empty hemithorax of pneumonectomized dogs caused the destruction of the superior mediastinal nodes in 90 per cent of those animals studied No harmful effects from this treatment were noted In this communication these results will be reviewed along with the results obtained by the mediastinal injection of the colloid at the time of pneumonectomy and following the intrapleural administration of the drug at the time of lobectomy

## METHOD

In these experiments mongrel dogs (weighing 10 to 19 5 kg) were used They were operated upon under aseptic conditions using intravenous barbiturate anesthesia At operation or at various times during the postoperative period radioactive silver coated gold colloid was injected into the pleural space or mediastinum The drug was obtained from the Brookhaven pile and processed after the method of Hahn[1] by the Abbott Laboratories of Chicago Illinois Previous studies[2] showed that it was necessary to use a dose of 40 mc or more in order to produce a consistent radiological effect on the mediastinal nodes The interval between operation and injection of the isotope did not alter the results The dogs were sacrificed at intervals ranging from 2 to 44 days after injection of the colloid material At the time of sacrifice representative lymph nodes were dissected out cleansed of adventitial tissue weighed and placed in standard formaldehyde tubes and the radioactivity determined by a Texas well counter Histologic studies of the right bronchial the carinal the left bronchial the anterior superior medi

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* From the Research Laboratory the Radioisotope Unit and the Pathology and Surgical Services Thayer V A Hospital the Cancer Research Laboratories of the Meharry Medical School and the Departments of Surgery Pathology and Medicine Vanderbilt University School of Medicine Nashville Tennessee

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*Table 2 Group 2 Dogs Receiving Mediastinal Infiltration of Gold Colloid*

| NUMBER | DOSAGE | $\beta$ SUPERIOR ANTERIOR MEDIASTINAL | $\beta$ SUPERIOR MEDIASTINAL LEFT | $\beta$ SUPERIOR MEDIASTINAL RIGHT |
|---|---|---|---|---|
| [illegible] | 50 mc | [illegible]1 700 | 12 013 | 18 [illegible] |
| 6 | 51 mc | 31 [illegible]00 | 16 700 | 169 800 |
| 7 | 51 mc | 1[illegible],200 | 32 000 | 76,200 |
| 8 | 51 mc | 69 000 | 10 500 | 2[illegible] 100 |
| 9 | 49 mc | 13 700 | 2 000 | 12 900 |
| 10 | 49 mc | 76 800 | 90 100 | 81 900 |
| 11 | 49 mc | 58 000 | 116 900 | 76 100 |
| 13 | 50 mc | [illegible]0 700 | 299,500 | 102 800 |
| 15 | 4[illegible] mc | 109 700 | 115 800 | 21 300 |
| 18 | [illegible]0 mc | 16,500 | 79 200 | 51 300 |
| 19 | 50 mc | 5[illegible] 800 | 8,200 | 204 500 |
| 22 | 50 mc | 40 100 | 184 000 | 114 400 |

The residual lung was inflated and the chest was closed without drainage These dogs were sacrificed from 7 to 10 days following the procedure Nine of the 10 dogs (90 per cent) had significant irradiation of the superior mediastinal group of nodes as determined by the Texas well counter (Table 3) On histologic study all the superior mediastinal nodes showed varying degrees of radiation effect There was 1 death in this series Dog No 21 died on the fourteenth postoperative day following a left upper lobe lobectomy At autopsy pneumonia was present in the left lower lobe and there was atelectasis on the contralateral side It was felt that this dog died as the result of the procedure but not as the result of the gold therapy Except in the dog that died the remaining lobe on the operated side was free of abnormalities at the time of autopsy

*Table 3 Group 3 Dogs Receiving Intrapleural Gold Colloid at Time of Lobectomy*

| NUMBER | DOSAGE | $\beta$ SUPERIOR ANTERIOR MEDIASTINAL | $\beta$ SUPERIOR MEDIASTINAL LEFT | $\beta$ SUPERIOR MEDIASTINAL RIGHT |
|---|---|---|---|---|
| 12 | 50 mc | 55 400 | 25 500 | 19 900 |
| 14 | 50 mc | 121,500 | 2[illegible]1 900 | Count too near background count |
| 16 | 4[illegible] mc | 81 000 | 93 100 | 40 800 |
| 17 | 50 mc | Count too near background count | Count too near background count | 6 100 |
| 20 | 50 mc | 112 600 | 110 900 | 180 100 |
| 21 | 50 mc | 191 400 | 6 300 | 36 300 |
| 23 | 50 mc | 223 800 | 84 000 | 2[illegible]6 600 |
| 24 | 50 mc | 91 [illegible]00 | 26 500 | 30[illegible] 200 |
| 2[illegible] | 35 mc | 177 000 | [illegible]57 000 | 389 700 |
| 26 | 3[illegible] mc | 290 000 | 219 000 | 384 300 |

astinal the right superior mediastinal and the left superior mediastinal lymph nodes were carried out Previous studies[2] revealed that a significant quantity of the colloid material was present in the mediastinal nodes of dogs sacrificed on the second postinjection day dogs sacrificed later did not have significantly more gold in the nodes studied Therefore, in calculating the beta equivalent roentgens it was assumed that irradiation emitted after 48 hr could be considered as the lymph node dose

## RESULTS

Of the 10 dogs in Group 1 that received more than 40 mc of the drug following pneumonectomy (Table 1) it is notable that the superior mediastinal group of nodes received adequate irradiation in 70 per cent of the cases In 2 dogs the interval between injection and counting was so long that the counts were not reliable Histologic study of these nodes however showed them to be markedly disorganized If these nodes are accepted as having received adequate irradiation the percentage is then 90 per cent *There was 1 death in this group of animals Dog No 18 died on the forty* fourth postoperative day of pneumonia which was thought not to be related to gold therapy This dog s bone marrow was normal All animals had well healed bronchial stumps at the time of autopsy

In the second experiment 12 dogs were subjected to pneumonectomy with the chest still open 45 to 51 mc (in 10 cc of solution) of radioactive silver coated gold colloid were injected into the mediastinum under direct vision All of these animals showed histologic destruction of the superior mediastinal group of nodes Radioactivity was also high as determined by the Texas well counter (Table 2) However 3 dogs (25 per cent) showed evidence of a bronchial stump fistula

In the third experiment 10 dogs were subjected to lobectomy At the time of operation 35 to 50 mc of the isotope were placed in the pleural space

*Table 1 Group 1 Dogs Receiving Intrapleural Gold Colloid Following Pneumonectomy**

| NUMBER | DOSAGE | β SUPERIOR ANTERIOR MEDIASTINAL | β SUPERIOR MEDIASTINAL LEFT | β SUPERIOR MEDIASTINAL RIGHT |
|---|---|---|---|---|
| 26 | 44 4 mc | 28 546 | 5 855 | 13 270 |
| 27 | 44 4 mc | 199 254 | 181 032 | 315 675 |
| 14 | 50 0 mc | 6,211 | 96 | 85 |
| 12 | 50 9 mc | 88 632 | 3 451 | 2 096 |
| 8 | 52 0 mc | 198 077 | 9 869 | 48 735 |
| 9 | 52 0 mc | 83,220 | 52 440 | 96 777 |
| 10 | 52 0 mc | 34 910 | 16 783 | Not counted |
| 18 | 75 0 mc | 150 314 | Count too near background count | Count too near background count |
| 19 | 75 0 mc | 365 489 | Count too near background count | Count too near background count |
| 28 | 150 0 mc | 94 620 | 86 549 | 181 716 |

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# INTRA OSSEOUS VENOGRAPHY *

## *Its Value to the Surgeon*

ROBERT SCHOBINGER VON SCHOWINGEN AND FRANZ P LESSMANN

By means of intra-osseous venography (I O V) certain venous pathways which hitherto partially or completely escaped visualization may be demonstrated The governing principle of this new technique is the injection of contrast medium directly into the medullary cavity of bony structures The diffusion of the so introduced contrast substance is on the one hand influenced by the hydrostatic pressure within the medullary cavity and on the other by the hemodynamic force within the systemic venous vascular bed The contents of the medullary cavity act as a poorly compressible column enclosed in rigid walls The injected contrast substance therefore diffuses under normal conditions over only a moderate distance within the medullary canal and chooses the path of least resistance This results in the prompt escape of contrast medium from the bone into the systemic venous circulation which rapidly transports the substance in centripetal direction

The following remarks are based upon our personal experience with I O V in over 300 patients

### METHOD

Local anesthesia is entirely sufficient Adequate sedation is obtained with thorazine (25 mg) demerol (75 to 100 mg) and seconal (0 1 gm) The former drug is administered 2½ hr and ½ hr the latter two 1 hr prior to the procedure The necessary precautions against idiosyncrasies to the local anesthetic agent or the contrast medium must be taken A 16 gauge Rosenthal bone marrow aspiration needle (BD #160 LNR) or the longer 16 gauge Lundy Irving needle (BD #161 LNRC) in case of trochanteric injection is introduced into the medullary cavity of the selected bone The correct positioning of the needle is verified by aspiration of bone marrow which may be saved for bone marrow smears The contrast medium used is 50 per cent Hypaque After introduction of 2 cc of contrast substance a scout film is taken verifying the correct position of the needle as well as of the patient Then 10 to 15 cc of Hypaque are injected under constant pressure except for the demonstration of the vertebral plexi where forceful and rapid injection is required Roentgenograms are taken during the injection of the last 2 to 3 cc of contrast medium Straining motion and forceful breathing by the patient should be avoided since increase of the intrathoracic or abdominal pressure (Valsalva maneuver) may result in poor or absent filling of certain veins The demonstration of the vertebral venous plexi is best accomplished by compressing the inferior vena cava with some radiolucent but firm material This temporary obstruction results in a shunt of venous blood over the vertebral plexi For more detailed information concerning method see Table 1

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The histologic changes in the mediastinal lymph nodes of all 3 groups can be considered together There was total necrosis of the nodes and the immediate surrounding tissues in those instances in which the radiological effect was the greatest In the less severe cases the nodal architecture was partially preserved, but with a complete loss of lymphocytes plasma cells and reticulum cells were prominent survivors in these nodes Erythrophagocytosis was noted in lymph nodes with high counts in animals that were sacrificed early It is notable that the vascular damage frequently assumed a form which strikingly resembles fibrinoid necrosis

DISCUSSION

One can conclude from these experiments that the lymphatics of the parietal and mediastinal pleurae in dogs drain into the mediastinal lymph nodes bilaterally These nodes may be destroyed by the intrapleural or intramediastinal injection of radioactive silver coated gold colloid It is felt that if these observations prove to be valid in the human a prophylactic type of mediastinal dissection may be added to pneumonectomy in the treatment of patients with resectable bronchogenic carcinoma Although we do not think it likely that lymph nodes replaced by tumor would pick up sufficient radioactive material to destroy them it is not unreasonable to believe that small tumor emboli in these nodes could be destroyed by this method We do not advocate the mediastinal injection of this drug in humans because of the high incidence of bronchial stump dehiscence in dogs

Six patients have been treated with radioactive silver coated gold following pneumonectomy for bronchogenic carcinoma at the Thayer Veterans Administration Hospital No ill effects have been observed from this therapy Dosage has ranged from 75 to 150 mc Four of these 6 patients are alive 4 to 26 mo following therapy without evidence of recurrent tumor

SUMMARY

1 Radioactive silver coated gold colloid consistently produces a radiologic effect upon the mediastinal lymph nodes of dogs following intrapleural *injection and following injection into the mediastinum*

2 No harmful effects have been noted following intrapleural administration The incidence of bronchial stump dehiscence following the intramediastinal injection of the colloid in pneumonectomized dogs was 25 per cent

3 Currently we are treating humans with the gold colloid following resective surgery for bronchogenic carcinoma We do not advocate the intramediastinal injection of the drug because of the high incidence of stump dehiscence in dogs

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Malignant lesions of the right lung, especially those located in the upper lobe, were in some instances found to produce a complete block of the azygos vein as evidenced by its lack of filling. Occasionally only partial obstruction or mere compression of the azygos vein was observed. All right-sided inoperable pulmonary neoplasms or cancers did present a complete block of the azygos vein. In the presence of some operable lesions, however, a lack of filling or compression of the azygos vein was, as verified during surgery, found to result from the huge size of the neoplasm itself or from enlarged azygos nodes. Benign or malignant lesions of the *left lung*, whether operable or inoperable, did not appear to obstruct the azygos vein. In one instance of an inoperable neoplasm of the left upper lobe involving the aortic arch, a mere displacement of a normal calibrated azygos vein to the right was observed. Hence the value of I O V in evaluating the operability of pulmonary lesions is, at the present time at least, difficult to assess. In other instances a previously blocked azygos vein may again be visualized after radiation therapy.

Esophageal neoplasms of all intrathoracic levels did, in a limited series of 6 cases, not appear to affect the course of the azygos vein. Mediastinal tumors associated with a superior vena cava syndrome caused obstruction of the azygos vein with deviation of the flow of contrast medium into distal veins or collateral channels such as the vertebral plexi, lateral thoracic and ascending lumbar veins.

Pelvic masses, be they the primary lesion itself, extension of the primary neoplasm or metastatic nodes, may lead either to complete obstruction, deviation or compression of the external and internal iliac veins. In case of pelvic venous obstruction a certain amount of collateral circulation over the presacral, iliac, sciatic, obturator and gluteal veins may be demonstrated. This is frequently associated with an increased filling of the femoral vein and occasionally with marrow stasis on the involved side. The origin and the type of malignancy appears to be less contributory to the degree of intrapelvic venous obstruction than the extent and location of the lesion. Chronic inflammatory changes other than pelvic phlebitis (we had no experience with this latter condition) and perhaps fibrotic changes have the tendency to distort the course of the veins and give their walls a ragged appearance, frequently without obstruction. On the other hand, complete lack of filling of the corresponding internal iliac vein was observed in 1 case of acute pelvic inflammatory disease and in 1 instance of tubal pregnancy (venous thrombosis).

Studies of the external and especially of the internal vertebral plexi of the dorsolumbar spine have been carried out in presence of metastatic, traumatic and degenerative vertebral lesions as well as in a few instances of disc protrusion. Interruption of the flow of contrast substance within the internal vertebral plexi was present in all our cases with metastatic neoplastic vertebral involvement, even in early lesions with normal myelograms. Occasionally there was also blockage of the ascending lumbar vein with distal deviation of the flow of contrast medium. Vertebral body disorders due to trauma or hypertrophic changes did not alter the pattern of the internal vertebral plexi on lateral views. Degeneration of the intervertebral disc may produce a variable degree of unilateral or bilateral blockage or none at all, depending upon the severity of the protrusion. These changes are much better

*Table 1*

| POSITION AND SITE OF INJECTION | AREA VISUALIZED |
|---|---|
| Antero posterior view Tenth rib in right mid axillary line Patient supine Contrast substance passes through the corresponding intercostal vein | Greater azygos vein |
| Lateral view Spinous process of $D_{11}$ or $D_{12}$ Patient in straight lateral position | |
| Injection similar to above but on the left | Hemiazygos vein<br>Collateral circulation in presence of portal hypertension |
| Midsternal line level of 4th rib Patient supine Syringe connected to needle by plastic tubing (bilateral visualization) | Internal mammary veins |
| Fourth or 5th rib of corresponding side anterior axillary line Patient supine (unilateral visualization) | |
| Acromial process head of humerus distal clavicle mid thoracic ribs in mid axillary line In the latter instance the substance flows over the lateral thoracic vein Patient supine | Subclavian vein |
| Spinous process $C_7$ Patient upright Postero anterior view of cervical spine | Vertebral veins |
| Mid axillary line of corresponding rib | Intercostal veins |
| Dorsal or lumbar spinous process of involved vertebra or of vertebra immediately below Compression of inferior vena cava Patient in straight lateral or prone position Latter provides much more detail | Internal and external vertebral plexi in area over 2 4 vertebrae corresponding to site of injection |
| Protuberances of peripheral bone | Deep and sometimes superficial venous circulation of corresponding extremity |
| Distal protuberances or distal end of shaft of long bones with additional needle in proximal end of bone in order to decrease the intramedullary pressure by escape of bone marrow through the proximal needle and allow ascension of contrast substance within shaft | Medullary cavity of long bones |
| Iliac crest greater trochanter pubic bone Patient supine Simultaneous bilateral injection provided best results The more medial the site of injection the more veins of the true pelvis are demonstrated | Pelvic system |
| Angle of mandible | Medullary cavity of mandible<br>Pterygoid plexus on corresponding side |

RESULTS

Review of examinations carried out in patients with benign or malignant lesions involving the lungs mediastinum esophagus pelvis breast spine long bones ribs and mandible and with portal hypertension varicose veins as well as prior and after radical neck dissections reveal that some observations are by necessity provisional while others are relevant of a definite pattern

Malignant lesions of the right lung, especially those located in the upper lobe, were in some instances found to produce a complete block of the azygos vein as evidenced by its lack of filling. Occasionally, only partial obstruction or mere compression of the azygos vein was observed. All right-sided inoperable pulmonary neoplasms or cancers did present a complete block of the azygos vein. In the presence of some operable lesions, however, a lack of filling or compression of the azygos vein was, as verified during surgery, found to result from the huge size of the neoplasm itself or from enlarged azygos nodes. Benign or malignant lesions of the left lung, whether operable or inoperable, did not appear to obstruct the azygos vein. In one instance of an inoperable neoplasm of the left upper lobe involving the aortic arch, a mere displacement of a normal calibrated azygos vein to the right was observed. Hence, the value of I.O.V. in evaluating the operability of pulmonary lesions is, at the present time, at least difficult to assess. In other instances, a previously blocked azygos vein may again be visualized after radiation therapy.

Esophageal neoplasms of all intrathoracic levels did, in a limited series of 6 cases, not appear to affect the course of the azygos vein. Mediastinal tumors associated with a superior vena cava syndrome caused obstruction of the azygos vein with deviation of the flow of contrast medium into distal veins or collateral channels such as the vertebral plexi, lateral thoracic and ascending lumbar veins.

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appreciated on postero anterior views since on straight lateral views the ascending lumbar veins may overlap the path of the internal vertebral plexi in the lumbar region By temporarily compressing the inferior vena cava with the patient in prone position myelogram like patterns of the internal vertebral plexi may be obtained and this without introduction of contrast medium into the spinal canal itself

Presently we are evaluating the flow through the vertebral veins before and after neck dissections in an effort to correlate the postoperative symptomatology with the degree of cephalic venous stasis especially in cases of bilateral neck dissection Preliminary information points to a poor, cephalo cervical venous drainage in cases with increased postoperative cephalic symptomatology as evidenced by the demonstration of narrow cervival veins in these patients as compared with normal or asymptomatic individuals

By means of venography of the internal mammary veins it is anticipated that better preoperative evaluation of the spread of breast carcinoma will be possible Up to the present time, 10 unselected cases with benign and malignant breast lesions have been screened with internal mammary venography In all instances where either deviation or obstruction of the internal mammary vein was noted a biopsy of that area was obtained Prominent retrosternal adipose tissue post irradiation pleural thickening abnormally narrow internal mammary vein segments and metastatic node involvement of variable degrees were found to be the cause of such venous alterations What appears established is that this type of examination may guide the surgeon to biopsy the correct site of possible metastatic spread and lead to a more precise therapeutic approach, be it by surgery or irradiation Furthermore the demonstration of possible retrosternal lymphatic spread in clinically cured patients may not only lead to a better evaluation of follow up results but also allow earlier institution of secondary therapy

The demonstration of an increased collateral venous circulation involving mainly the intercostal veins and vertebral plexi in patients with portal hypertension and with or without radiologically demonstrable esophageal *varices, may prove to be of definite diagnostic and therapeutic value* to the surgeon It is conceivable that demonstration of such collateral systemic venous channels in patients with upper gastrointestinal hemorrhage of unknown origin would point toward the probability of bleeding varices rather than of a bleeding ulcer However this promising avenue which we recently entered must be more thoroughly exploited before definite conclusions can be reached one of them being that the demonstration of such a collateral circulation in an early cirrhotic but otherwise asymptomatic patient could allow shunt procedures to be performed at a much earlier and therefore favorable time

Intra osseous venography is advantageously used in the demonstration of the deep venous circulation of the leg since the flow of the introduced contrast substance is into the deep systemic veins Better evaluation of deep varicose veins and associated disorders such as the localization of a phlebitic obstruction may be carried out without contraindication since the contrast substance is not introduced directly into the venous circulation The superficial veins may or may not be visualized by this method but failure to visualize the deep veins speaks in itself unquestionably for a block

The uniformity of intramedullary diffusion of contrast medium may be

altered by certain disorders. The present study was mainly concerned with the detection of early metastatic lesions in the ribs. Such neoplastic deposits were found to obstruct the intramedullary propagation of contrast medium with concomitant formation of collateral channels in the area of the lesion. Principally the same approach may be utilized in studying the intramedullary circulatory pattern in the presence of benign or malignant conditions in other locations. Our present investigations in this regard comprise studies concerning the local extent of tumors of the jaw, localization of tumors of peripheral bones and circulation in fractures.

SUMMARY

The authors have submitted their observations as obtained by means of intra-osseous venography. The demonstration of certain hitherto hidden venous pathways such as the study of the internal mammary, azygos and pelvic veins as well as vertebral plexi proved to be of definite value to the surgeon. The great potentialities, the simplicity of technique, and the relative safety of intra-osseous venography certainly invite further investigative work.

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# THE CIRCULATION OF BONE WITH PARTICULAR REFERENCE TO THE MEDULLA AND MEDULLOGRAPHY*

*Intraosseous Phlebography*

RUTHERFORD S. GILFILLAN, NICHOLAS L. PETRAKIS
AND HOWARD L. STEINBACH

The circulation in the hemopoietic bone marrow is a dynamic one in direct communication with the extraosseous systemic circulation as manifested by pressure measurements,[1] clearance of isotopes[2] and opaque media. Study of the medullary pressure reveals a pulse wave as well as respiratory fluctuation and response to vasoconstrictors such as adrenalin. The Valsalva maneuver (forced expiration against a closed glottis) is followed by a prompt rise

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Fig 1 Vinylite injection of the arterial system of a 5 mo fetus A Nutrient artery of tibia B Sinusoids filled in upper diaphysis and metaphysis of tibia C Small branches of geniculate artery forming metaphysial arteries D Nutrient artery vein and sinusoids outlining distal diaphysis and metaphysis of femur

in intramedullary pressure and the Mueller maneuver (forced inspiration against a closed glottis) produces a drop in pressure Injection of $I^{131}$ into the marrow cavity is followed by a clearance rate approximately similar to that of muscle Injection of contrast media (Hypaque) into the ends of long bones shows its removal to be more rapid than that from muscle or other soft tissue The ease and rapidity with which large quantities of fluid can be introduced into the marrow demonstrates not only the capacity of the venous outflow tract but also suggests that the introduction of opaque media in a similar fashion might provide a radiographic means of demonstrating the venous drainage and visualization of the veins of the surrounding soft tissues

Knowledge concerning the morphology of the circulation of bone in man and animal has been derived from the analysis of the work of Doan[3] Drinker[4] and Trueta[5] and by the careful inspection of actual injections of vinylite followed by corrosion in fetuses and stillborn infants (Fig 1) Direct injection of amido trizoate sodium (Hypaque) 50 per cent into the medullary canal of bone affords further data concerning the flow within the bone and its venous drainage

The arterial supply of cortical or compact bone is derived from the numerous vessels ramifying in the periosteum and connecting with the longitudinal Haversian canals through the horizontal canals of Volkmann This supply also is in continuity with the vessels of the medullary canal The cancellous or spongy bone is supplied by less numerous but larger vessels the numerous openings for which are readily visible near the articular surfaces at the ends of long bones or in relation to musculo tendenous attach



Fig 2 Foramina for transmission of vessels in the condyles of the femur (left) and in the anterior tibia just below the plateau (right)

ments (Fig 2) Some of these openings transmit arteries but the majority of them form a path for emergence of veins A third supply is that of the nutrient artery and vein or veins which are usually seen entering the shaft of a long bone and pursuing an oblique course through the cortex to divide on reaching the medullary canal proximally and distally to anastomose with the preceding described sources of blood flow These vessels during development might be considered as the blood supply of the diaphysis and end vessels to the metaphysis The nutrient artery and vein become smaller with increasing age and development because of the decreased blood supply necessary in the shaft as a result of replacement of the active hemopoietic bone marrow with the less vascular fatty marrow The sinusoids for the most part are flat ovoid spaces connected at two or three points to adjoining spaces or vascular channels The sinusoids of the epiphysis and the diaphysis are in direct continuity following the cessation of growth and the disappearance of the epiphyseal plate

## METHOD

The injections in this series were carried out with a standard Turkel bone marrow needle with trepine under either local or general anesthetic The injection of the contrast media sometimes proved to be extremely painful because of the high pressures exerted in the initial phases of the injection On 2 occasions the appearance of pain was correlated with the pressure exerted by attaching a flask of saline to the needle and elevating it above the injection site at the malleolus of the tibia Discomfort was not noted until the flask was elevated *290 cm* above the tibia *on the first occasion and 300* cm on the second occasion Because of the pain a short acting general anesthetic was commonly used

The needle was inserted into the distal end of a long bone or directly into a flat bone with a rotary movement One to 1 cc of bone marrow was aspirated followed by the injection of 10 to 20 cc of an aqueous contrast media Hypaque 50 per cent was the contrast media used in all instances in this study The first roentgenogram was usually exposed immediately after the completion of the injection Additional roentgenograms were obtained at suitable intervals which varied depending upon the purpose of the examination The use of a plastic tube connecting the syringe to the bone marrow needle was of value




Fig. 1. Vinylite injection of the arterial system of a 5 mo. fetus. A. Nutrient artery of tibia. B. Sinusoids filled in upper diaphysis and metaphysis of tibia. C. Small branches of geniculate artery forming metaphyseal arteries. D. Nutrient artery, vein and sinusoids outlining distal diaphysis and metaphysis of femur.

in intramedullary pressure and the Mueller maneuver (forced inspiration against a closed glottis) produces a drop in pressure. Injection of $I^{131}$ into the marrow cavity is followed by a clearance rate approximately similar to that of muscle. Injection of contrast media (Hypaque) into the ends of long bones shows its removal to be more rapid than that from muscle or other soft tissue. The ease and rapidity with which large quantities of fluid can be introduced into the marrow demonstrates not only the capacity of the venous outflow tract but also suggests that the introduction of opaque media in a similar fashion might provide a radiographic means of demonstrating the venous drainage and visualization of the veins of the surrounding soft tissues.

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## DISCUSSION

The contrast material injected into cancellous bone presents a homogeneous poorly delineated shadow in the roentgenogram which persists after the major portion of the media has drained to the systemic extra osseous veins The majority of the injection mass passes rapidly into small interlacing channels which rapidly converge to form larger trunks to emerge from the bone and fill the deep and superficial soft tissue veins (Fig 3) Injection into long bones is usually followed by filling of the medullary vein for approximately one third of the length of the shaft The so called medullary vein is a tortuous irregular channel sometimes presenting the appearance of a group of communicating sinusoids The medullary vein may be more prominent with the presence of a tumor probably due to increased flow in



Fig 7 Nodular vasculitis of both legs Injections into the tibial malleolus of both legs with filling of deep and superficial veins of leg and thigh



Fig 8 Deep and superficial thrombophlebitis Injection into medial condyle of the femur Femoral vein is obstructed and the thrombosed saphenous vein is not visualized Marked edema was present



Fig 9 Multiple myeloma Injection into the ilium with visualization of the superior gluteal and hypogastric vein




Fig 3 Old non union fracture of distal one third of tibia and fibula Injection into calcaneus 20 cc. 50% Hypaque The tortuosity and irregularity in the region of the old fracture are due to the venous damage at the time of fracture A At completion of injection B At 15 seconds C At 45 seconds



Fig 4 Osteogenic sarcoma shaft of left femur Injection of 50% Hypaque into lateral condyle Medullary vein is large irregular and tortuous The media passes readily to the soft tissue tumor mass



Fig 5 Cavernous venous hemangioma of vastus medialis residual tumor demonstrated following surgical removal Injection site medial condyle of femur




Fig 6 Varicose veins of the left leg Injection of 50% Hypaque into tibial malleolus The contrast media spread across the epiphyseal line outlining the deep and superficial circulation There is a perforating vein visible at mid shaft communicating with a vein posterior to the saphenous vein

# AERIAL METASTASIS OF TUMORS IN THE LUNG AN EXPERIMENTAL STUDY IN MICE*

TOM D NORMAN AND ROBERT D McBROOM

The possibility of aerial metastasis of tumors within the lung has been largely overlooked in clinical and experimental cancer investigations. Isolated examples of this route of tumor spread in man have been reported but these cases have not been conclusive enough to establish it definitely as a mechanism of tumor dissemination.

Experimental evidence of the role of the bronchi in tumor spread was reported by Furth[1] in 1916: tumor cell suspensions placed into the nares of mice resulted in pulmonary tumors in a significant number of animals. The tumors used were a mouse lung carcinoma and leukemias.

The present investigation was initiated in order to study the incidence and morphology of lung tumors in the mouse resulting from the injection of tumor cell suspensions directly into the trachea as well as into sites adjacent to the upper respiratory passages.

## METHODS

$C_3H$ mice of mixed sex were used in all of the experiments; these animals were supplied with Purina mouse food and water *ad libitum*. Tumors used were a mammary adenocarcinoma ($C_3HBA$), a hepatoma (98/15) and a fibrosarcoma (HL 8971).[2]

Tumor cells were suspended in normal saline in a concentration range from 10 to 50 per cent. Mice were divided into groups for injection of the tumor cells as follows: intratracheal (mammary tumor) 10 mice, intratracheal (hepatoma) 9 mice, intratracheal (fibrosarcoma) 12 mice, submucosa of mouth (mammary tumor) 13 mice, and submucosa of nares (mammary tumor) 6 mice. With the mice anesthetized by intraperitoneal pentobarbital the intratracheal injections were made by insertion of a dull 20 gauge needle through the mouth and larynx into the lumen of the trachea. Placement of the needle in the trachea was checked by inspection through an incision anterior to the trachea. The amount of cell suspension injected ranged from 0.05 to 0.1 ml, but most of the mice received 0.05 ml. Some of the mice which had submucosal injections of tumor cells in the mouth were anesthetized and had incisions made over the tumor once or twice during its development. Except for the few mice that died immediately after injection, the interval between instillation of the tumor cells and death or sacrifice of the animal was as follows: intratracheal (mammary tumor) 22 to 33 days, intratracheal (hepatoma) 31 to 91 days, intratracheal (fibrosarcoma) 14 to 32 days, submucosa of mouth 10 to 18 days, and submucosa of nares 12 to 17 days. At autopsy the lungs from each mouse were examined for gross tumors, fixed in 10 per cent formalin, imbedded in paraffin, and multiple sections stained with hematoxylin and eosin for microscopic examination.

## RESULTS

**Intratracheal administration.** Of the 10 mice injected with the mammary tumor 3 died immediately after receiving the cell suspension. Seven mice

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the region of the tumor[4] (Fig 4) Other tumors such as hemangioma of the adult venous type are easily visualized (Fig 5) Injection of the epiphyseal region in the adult is followed by spread of the contrast media across the epiphyseal line (Fig 6) This does not occur during the active growing period

Injections into the tibia, fibula femur, and calcaneus fill the deep and superficial veins making possible the visualization of the perforating veins For this procedure the patient should be tilted with the legs dependent (Fig 7) This is of value in an edematous and ulcerated extremity or in an extremity where thrombosis of superficial veins is feared (Fig 8) The veins of the pelvis may be visualized by injection of greater trochanter of the femur the pubis or the iliac crest (Fig 9) The azygos system can be visualized via the vertebral body or spinous processes

SUMMARY

This method of examination is useful in the study of veins in relation to

1 Thrombosis and varicosis

2 The delineation of tumors and vascular alterations produced by them

3 The study of the anatomic and pathologic status of bone and hematopoiesis

Its disadvantage is that of requiring anesthesia Slower injection rates may obviate the necessity for anesthesia

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produced in the lungs were identical morphologically to the tumors used to prepare the cell suspensions

The reasons for the much lower rate of successful pulmonary implantation with the hepatoma and fibrosarcoma are not clear and this phase of the problem needs further study The only explanation offered is that in the case of the hepatoma the percentage of takes subcutaneously is considerably less than with the other tumors used that tumor is also much slower growing than the other neoplasms

The few tumors that occurred in the lungs of the mice that had injections of tumor cells into the submucosa of the mouth were evidence that sloughing tumor cells can be disseminated through the air passages to produce metastatic lesions The protrusion of the tumors into the mouth with ulceration of the overlying mucosa gave support to that concept No proof can be given that the lung tumors did not occur by lymphatic dissemination from the primary transplant and one of the animals especially raises the possibility of such spread since the tumor developed in the mediastinal pleural and subpleural regions However the absence of tumor in all lymph nodes examined is evidence against a lymphatic type of metastasis It was planned to include control animals with injections subcutaneously in the cheek however it became apparent that with growth of the tumor the soft tissue of that general area was involved extensively without regard to the exact point of injection Studies are planned using injections subcutaneously in the neck to rule out the possibility of lymphatic metastasis to the lung This experiment was made difficult by the fact that survival time of the mouse after development of a tumor in the mouth region is less than the period necessary to develop tumors of even very small size following cell dissemination through air passages

## SUMMARY

Metastatic lung tumors have been produced in the $C_3H$ mouse following intratracheal instillation of cell suspensions from 3 tumors a mammary adenocarcinoma ($C_3HBA$), a hepatoma (98/15) and a fibrosarcoma (HE 8971) The mammary tumor developed in the lung much more readily than did the other 2 neoplasms

Four tumors developed in the lung after the submucosal transplantation of the mammary tumor in the mouth It is believed that this represents development of tumor from the sloughing of tumor cells and their dissemination through the air passages but the possibility of lymphatic spread cannot be absolutely ruled out as the responsible mechanism

It is concluded that these experiments support the concept of aerial dissemination of tumors

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Fig 1 Gross tumor nodules in the lungs of a mouse in which a suspension of the mammary tumor cells had been injected intratracheally



Fig 2 Tumor in bronchiole of the lung shown in Figure 1

developed multiple pulmonary tumors, the gross appearance of which is illustrated in Figure 1 The size of these tumors varied roughly with the length of the post injection period

Injection of the hepatoma cell suspension into the trachea resulted in 1 tumor of microscopic size 4 of these animals did not survive the initial inoculation of the tumor cells Two of the mice injected with the fibrosarcoma died immediately 1 of the remaining 10 mice developed gross tumors

Microscopically these tumors could be seen filling bronchi and bronchioles *(Fig 2) and extending out into adjacent lung tissue In some areas* masses of tumor not related to visible bronchi were noted Cell detail of the tumors did not differ from that seen in these neoplasms in subcutaneous type of implants

**Submucosal injection (mouth)** Only the mammary tumor was used for these studies Of the 43 mice inoculated with the tumor cells 9 died immediately Of the remaining animals 4 developed pulmonary tumors all of them discernible only by microscopic observation Two of the lung tumors occurred in animals whose mouth tumors were incised during their development *In 1 animal the tumor was subpleural and pleural in location* and tumor was also present in the mediastinum All of the tumors were identical to the primary tumors in the soft tissues of the mouth

**Submucosal injection (nares)** None of these mice died after injection and none developed tumors

DISCUSSION

The tumors which developed in the lungs following the intratracheal injections were very definitely growing out of bronchi It was felt that control *animals injected with material other than tumor* cell suspensions were not necessary for the following reasons spontaneous tumors have not been noted in these mice at the age range used and both spontaneous pulmonary tumors and induced non neoplastic proliferative disease can easily be separated histologically from these tumors There was no doubt that the tumors

*Table 1 Challenge Dose of 20 Million Tumor Cells*

| NO. MICE | TREATMENT | NO. DIED | MEAN SURVIVAL (DAYS) |
|---|---|---|---|
| 10 | Antiserum | 10 | 14.8 |
| 10 | Controls | 10 | 10.8 |
| 10 | Lymphocytes | 10 | 14.2 |
| 10 | Controls | 10 | 10.6 |

bled and the serum collected. At the same time the spleen was removed and a washed suspension of spleen cells in balanced Ringer's solution was prepared.

The antiserum and lymphocyte suspension were stored at about 2° C until injected. Penicillin and streptomycin in small quantities were added to each solution. The longest each solution was stored prior to injection was 4 days.

Susceptible mice were injected with viable tumor cells, the size of the challenge dose being computed by means of hemocytometer counts of single cell suspensions of the tumor. Treatment was instituted with either antiserum or lymphocyte suspension within 12 to 18 hr.

In the first group (Table 1) all mice were challenged with a large dose of tumor cells (20 000 000). They were divided into 4 groups. One group was selected to be treated with antiserum and another to receive the lymphocyte suspension. The remaining 2 groups served as controls. Those receiving antiserum were injected daily with 0.1 cc subcutaneously for 6 days. The group given the lymphocyte suspension received 0.2 cc of the suspension daily for 6 days.

In the second group (Table 2) the challenge dose of tumor cells varied from 10 000 to 1 000 000. Antiserum alone was used for treatment in this group. The dosage schedule was the same as for the first group. Each of the 3 subgroups had control counterparts.

RESULTS

It may be noted in Table 1 that when a large dose of tumor cells was injected, all animals in both the control and treated groups succumbed to the disease, but there was a definite increase in survival time in the treated groups.

Table 2 demonstrates the complete protection afforded by the antiserum when the challenge dose of tumor cells is minimal.

*Table 2*

| NO. MICE | TUMOR CELL DOSE | TREATMENT | NO. DIED |
|---|---|---|---|
| 12 | 10 000 | Antiserum | 0 |
| 12 | 10 000 | Controls | 10 |
| 12 | 100 000 | Antiserum | 0 |
| 12 | 100 000 | Controls | 12 |
| 12 | 1 000 000 | Antiserum | 2 |
| 12 | 1 000 000 | Controls | 12 |

# TREATMENT OF EXPERIMENTAL LYMPHOID TUMOR WITH HETEROLOGOUS ANTISERUM AND IMMUNE LYMPHOCYTES*

JAMES T. GRACE, FRANK GOLLAN AND ROBERT J. CARLSON

In recent years there has been a considerable revival of interest in the immunologic approach to cancer research.

Early reports of the use of antisera prepared against malignant tissue in the treatment of experimental cancer showed equivocal results. Possible explanations for these failures have been discussed by Pressman.[7]

Numerous other studies have demonstrated that tumor specific antibodies can be produced in the homologous or heterologous animal by injection of either whole tumor cells or certain fractions thereof. Furthermore, the cytotoxicity of such antibodies for the specific tumor cell has been amply shown. Incubation of viable tumor cells with such antiserum prior to injection into the susceptible animal prevented the production of the disease.

More recently, Gorer and Amos[1] have shown that mice could be afforded considerable protection against inoculation with a strain susceptible leukosis by pre-treatment with iso-immune serum.

This study was undertaken to assess the value of hyperimmune heterologous serum on the course of a mouse leukosis when treatment with the antiserum was begun after inoculation with the disease.

No attempts were made to remove antibodies from the antiserum which may have been formed against normal tissue components by absorption or neutralization techniques.

It was further desired to note also the effect of the use of cellular elements from the heterologous animal. For this purpose a washed suspension of spleen cells was employed.

## METHOD

The malignant disease used in this study was the P1534 leukosis carried in the DBA/2 strain of inbred mice.† Within 5 to 7 days after inoculation subcutaneously with this type tumor cells there appeared a palpable tumor at the site of injection. This rapidly progressed to a typical lymphatic leukemia which was fatal in 10 to 11 days when a standard tumor cell dose inoculum was used.

*The standard laboratory white rabbit was employed* for the production of antiserum and the antigen consisted of a suspension of spleen cells from mice moribund with the disease.

The rabbits were injected initially intraperitoneally with 2 cc of an emulsion consisting of equal parts of antigen and Freund's adjuvant. The amount of splenic tissue injected was equal to approximately 100 mg of wet weight. Subsequently, 2 additional injections of such an emulsion were made at 2 wk intervals, these being given subcutaneously at multiple injection sites. Two weeks following the last injection, a final boost of antigen in saline was given subcutaneously. Ten days after this the animals were

*From the Research Laboratory and Surgical Service, Thayer Veterans Administration Hospital, and the Department of Surgery, Vanderbilt University School of Medicine, Nashville, Tenn.

†Obtained from Jackson Memorial Laboratory, Bar Harbor, Maine.

considered the result of an autotransplantation of tumor cells detached from the primary growth it was thought that the host might show a corresponding increase in susceptibility toward a reinoculation of the same tumor concomitant with an increasing size of the primary

## METHOD

To test this hypothesis a series of experiments was conducted in the following way $Z(C_3H)$ back cross hybrid mice (ZBC) were used as recipient hosts The tumor employed was a transplantable mammary adenocarcinoma originally arising in a $Z(C_3H)$ breeder mouse which had been maintained in this laboratory for 53 successive transfers into mice of the $Z(C_3H)$ strain $F_1$ hybrids and ZBC back-cross hybrids without losing its original genetic characteristics since it does not grow in any strain other than those listed

Mice were first inoculated with this tumor into the subcutaneous tissue of the tail by injecting 0 05 cc of a tumor cell suspension prepared as an 8 per cent concentration in saline At 12 17 and 22 days after transplantation groups of mice growing caudal tumors were submitted to amputation of their tails by clipping them off near the root of the tail with a pair of hot sterilized scissors The removal of the tail was followed in each mouse by the subcutaneous inoculation into the right groin of 0 25 cc of a second tumor suspension prepared as a 5 per cent concentration The tumor used for the second inoculum was taken from the same group of donors used for the original tail transplant

Each group of experimental animals had its own controls consisting of mice receiving the second transplant after clipping off their normal tails After the groin implant mice were inspected once every other day for the appearance of subcutaneous tumors at the site of inoculation and the progressive incidence of takes determined in the various groups

## RESULTS

Tables 1 and 2 show the results obtained in 2 similar experiments As can be seen (Table 1) the second inoculation made at 12 days after the tail transplant produced new tumors earlier than in its controls although the final tumor incidence in both groups was almost the same (92 and 90 per

*Table 1 Incidence of subcutaneous tumors obtained in mice reinoculated at different time intervals after a prior caudal transplant*

| TIME BETWEEN 1ST AND 2ND TRANSPLANT (DAYS) | GROUP | NUMBER OF MICE | PROGRESSIVE INCIDENCE OF SUBCUTANEOUS TUMORS AFTER THE SECOND IMPLANT (PER CENT) DAY 12 | 16 | 20 | 22 |
|---|---|---|---|---|---|---|
| 12 | Exper | 37 | 3 | 76 | 92 | 92 |
| | Controls | 33 | 0 | 30 | 85 | 90 |
| 17 | Exper | 27 | 11 | 37 | 56 | 56 |
| | Controls | 30 | 13 | 37 | 73 | 93 |
| 22 | Exper | 28 | 0 | 0 | 7 | 7 |
| | Controls | 28 | 7 | 43 | 79 | 93 |

### DISCUSSION

These studies demonstrate the ability of heterologous antiserum and spleen cell suspensions to influence the course of a malignant disease in the mouse When a large tumor cell inoculation was followed by administration of relatively small quantities of these treatment materials all animals succumbed to the disease but showed a definite prolongation of survival time as compared to controls However, when the tumor cell dose was markedly reduced and the antiserum doses remained the same it was possible to arrest the disease in practically all cases This suggests that there is a direct relationship quantitatively between the number of tumor cells present and the amount of antiserum administered If this simple relationship does exist it would appear that merely by increasing the number of antibodies more advanced disease could be controlled

There are many problems which require further study It is planned to evaluate the effect of larger doses of antiserum in the presence of more advanced disease Additionally it is hoped that fractionization of serum proteins will yield a more concentrated solution of antibodies Further study of the nature of the antigen and improved immunization procedures should produce higher antibody titers in the serum

### SUMMARY

Serum and lymphocytes from rabbits which were immunized with P1534 mouse leukosis favorably alter the course and mortality of the disease in susceptible mice when treatment is begun 12 to 18 hr after inoculation with viable tumor cells

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## IMMUNITY IN MICE TO STRAIN SPECIFIC TUMOR TRANSPLANTATION FOLLOWING GROWTH AND REMOVAL OF THE SAME TUMOR*

CARLOS MARTINEZ J BRADLEY AUST AND JOHN J BITTNER

It has previously been established that the incidence of lung metastases in mice growing a transplanted tumor is related to the size of the primary i e mice bearing large growths exhibited more metastases than those bearing smaller tumors (Wood *et al* [4] Martinez *et al* [3]) Since metastases may be

*Departments of Physiology and Surgery University of Minnesota Medical School Minneapolis Minn Aided by grants from the Minnesota Division of the American Cancer Society and the National Public Health Service C 2168

experiments was able to induce immunity in mice reinoculated with Sarcoma 180 when tested 2 wks after the first transplant His results were confirmed by one of us (J J B[2]) for Sarcoma 180 in some inbred stocks of mice but not in others However he was unable to produce immunity when using a 7($C_3H$) tumor retransplanted into mice of the same strain $F_1$ hybrids or 7BC hybrids 2 wks after caudal inoculation Our data are in agreement with these later results since using a similar tumor and the same type of mice no immunity was observed when the test was performed at 12 days However at 17 and especially at 22 days immunity to the second inoculation was clearly established To our knowledge this constitutes the first instance in which immunity could be demonstrated by using inbred stock mammary tumors

The nature of the immunity observed in these experiments has not been clarified since preliminary attempts to transfer this immunity to normal mice using serum or splenic tissue of the resistant animals have been unsuccessful

## SUMMARY

Experiments in mice to test host susceptibility to a second inoculation of an inbred mammary tumor made at various intervals following a tail tumor transplant have shown the following results a) if the second inoculation was made at 12 days after the caudal transplant an acceleration in the take of the tumors in the experimental group as compared to the controls was observed b) if the reinoculation was made at 17 and particularly at 22 days a high degree of resistance was observed This has been interpreted as due to some form of immunity induced in the host by the continuous growth of the caudal tumor

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*Table 2. Incidence of subcutaneous tumors in mice reinoculated at different time intervals after a prior caudal transplant*

| TIME BETWEEN 1ST AND 2ND TRANSPLANT (DAYS) | GROUP | NUMBER OF MICE | PROGRESSIVE INCIDENCE OF SUBCUTANEOUS TUMORS AFTER THE SECOND TRANSPLANT (PER CENT) DAY 12 | 16 | 20 | 24 |
|---|---|---|---|---|---|---|
| 12 | Exper | 30 | 60 | 87 | 97 | 97 |
| | Controls | 30 | 50 | 72 | 92 | 100 |
| 17 | Exper | 39 | 0 | 9 | 10 | 19 |
| | Controls | 27 | 4 | 30 | 67 | 93 |
| 22 | Exper | 20 | 0 | 0 | 0 | 0 |
| | Controls | 23 | 10 | 26 | 61 | 87 |

cent respectively). The transplant made at 17 days after the tail inoculation produced less tumors than in the control group (56 and 93 per cent respectively) the difference being statistically significant. Finally in the group transplanted at 22 days only 7 per cent of the experimental group developed tumors as compared to 93 per cent of the controls.

The results of a second experiment done under the same experimental conditions gave qualitatively the same results (Table 2).

It is interesting to point out that most animals in the 17 and 22 day groups had metastases in their lungs when sacrificed at the end of the experiment. However there were no signs of malnutrition or cachexia in mice having metastases since the determination of the mean body weight in both experimental and control mice of the 22 day group did not reveal significant differences.

DISCUSSION

The results of these experiments indicate that with the exception of the group reinoculated 12 days after the first transplant, which showed an acceleration in the takes, the groups reinoculated at 17 and 22 days exhibited a marked resistance. The acceleration observed might be interpreted as representing an enhanced susceptibility to tumor growth. In this respect it is interesting to point out that the time at which the host seems to be hypersusceptible to isotransplantation is coincident with the time of initiation of the metastatic spread of the first tumor. This is consistent with observations reported in previous studies (Martinez *et al*[3]).

The basis for the resistance observed when mice were reinoculated at 17 and 22 days after the caudal transplant is not clear. Most of the resistant animals exhibited lung metastases. The question arose as to whether or not the presence of metastases in the lungs could interfere in some way with the take of the second tumor. However in recent experiments to be published elsewhere the same phenomenon was obtained in animals in which metastases from the first tumor were prevented. This would indicate that the presence or absence of metastases in the host does not play any role in the development of resistance to tumor reimplantation. On the other hand it is conceivable that the continuous growth of the caudal tumor could have induced some form of immunity. In fact Andervont[1] in similar

*Table 1 Growth of Tumor Homografts in Mice*

| TUMOR | HOST | # ANIMALS | # TAKING | % |
|---|---|---|---|---|
| Z | Mature A | 9 | 0 | 0 |
| Z | Newborn A | 17 | 9 | 53 |
| Z | Newborn Cc | 15 | 12 | 80 |
| A | Newborn Z | 8 | 8 | 100 |

*Table 2 Excision and Retransplantation of Tumor Homografts Growing in the Newborn at the Time of Weaning*

| TUMOR | HOST | # | # REGROWING TUMOR |
|---|---|---|---|
| A | $D_8$ | 8 | 0 |
| Z | $D_4$ | 6 | 0 |

*Table 3 Histocompatibility Characteristics of Z Tumors Grown in A Mice*

| STRAIN | # MICE TREATED | # TAKING TUMOR |
|---|---|---|
| A | 9 | 0 |
| Z | 9 | 9 |

lung metastasis at post mortem Similar results were obtained using Z tumor into newborn Cc mice and A tumor into newborn Z mice with up to 100 per cent of the mice taking the tumor In the earlier studies tumor suspension leaked from the site of needle puncture and may have accounted for the higher per cent of takes later when the needle site was coated with collodion Groups of $D_8$ mice treated similarly with Z and A tumors in the newborn period took the tumors At the time of weaning these tumors were removed homogenized a 10 per cent suspension prepared in saline and 5 ml reinjected into the same animal from which it was excised Table 2 shows that the tumor failed to regrow in all instances indicating the absence of acquired tolerance at that time

To test whether the Z tumor growing in an A mouse retained its normal histocompatibility characteristics the following experiment was carried out The Z tumor which had grown in an A mouse for 1 mo was excised a 5 per cent tumor suspension in saline prepared and 25 ml injected into the groins of 9 adult Z and 9 adult A mice As is seen in Table 3 the tumor grew only in the Z mice indicating that it did retain its normal histocompatibility characteristics despite a period of growth in an A host

## DISCUSSION

These findings demonstrate that tumor homografts may be successfully transplanted between highly inbred strains of mice provided such a procedure is carried out in the first few hours of life The mechanism responsible for this is not completely clear Based on observations that some rats

# TUMOR HOMOTRANSPLANTATION IN INBRED MICE DURING THE NEWBORN PERIOD*

J. BRADLEY AUST, CARLOS MARTINEZ, JOHN J. BITTNER AND ROBERT A. GOOD

It is an established fact that tumors arising spontaneously in one highly inbred strain of mice will not grow in an animal of another highly inbred strain. This rejection of tumor homografts in inbred strains of mice has been shown to be a function of the genetics of histocompatibility[1] and is identical in mechanism to homograft rejection.[2]

Recently Billingham[3, 4] succeeded in producing a state of "acquired tolerance" in mice characterized by their ability to accept homotransplants of skin from donors whose tissues had been implanted into the recipient during the embryonic period. Interest in this phenomenon had been stimulated by Owen who discovered that dizygotic twin cattle may continue to circulate blood cells of 2 distinct genotypes (their own and their partner's). Reciprocal skin homografts will grow between such chimera, but they will not accept skin homografts from their mother, siblings, or other twin cattle.[6, 7] The ability to produce acquired tolerance following introduction of living foreign cells in the mouse is optimal in the embryo and becomes gradually lost as birth approaches.[4] Woodruff,[8] studying the rat, found that varying degrees of "acquired tolerance" could be produced up to 2 wks after birth.

This report cites an attempt to produce "acquired tolerance" in inbred strains of mice by implantation of tumor homografts in the neonatal period. The results indicate that such homografts will take and grow progressively. However, following removal of these tumors, the animals fail to exhibit acquired tolerance.

## METHOD

*Mice of the A, Z (C$_3$H), Ce and D$_8$ strains were used.* A transplantable Z mammary carcinoma of the 55th transplant generation and a transplantable A mammary carcinoma of the 41st transplant generation were the tumors employed. Each tumor grows only in members of its own specific strain. Fresh 5 per cent tumor concentrations in saline were prepared using a Potter-Elvejhem glass homogenizer. Mice of an homologous strain were injected subcutaneously under the nape of the neck before reaching 3 hr. of age. At 1 mo. of age the mice were weaned and the incidence of tumor was determined. Following this some of the tumors were excised, homogenized and reinjected into their respective hosts.

## RESULTS

Table 1 confirms that the Z tumor used would not grow in mature A mice. However, when the tumor was implanted into newborn A mice, over half of the mice grew the tumor progressively to death and exhibited

*Departments of Surgery, Physiology and Pediatrics, University of Minnesota Medical School, Minneapolis, Minn. Aided by grants from the Minnesota Division of the American Cancer Society, the United States Public Health Service Grant C-2168, and the Helen Hay Whitney Foundation.

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DISCUSSION

These findings demonstrate that tumor homografts may be successfully transplanted between highly inbred strains of mice provided such a procedure is carried out in the first few hours of life The mechanism responsible for this is not completely clear Based on observations that some rats

previously challenged during neonatal life with splenic tissue accepted one skin homograft but rejected subsequent grafts from the same animal. Woodruff postulated a so called critical period. He suggested that the homograft took during a period of partial tolerance and was resident in the host for a critical period long enough for the graft to adapt to the host and grow thereafter. Subsequent grafts at a time when tolerance had disappeared were rejected before such adaptation could occur. If this concept applies to our studies using tumor homografts, the original tumor when excised and reimplanted into the same host might be expected to grow again since it had already adapted to the host. However, this was not found to be true and all reimplantations of tumor failed.

The neonatal period represents a time when immunological capacity is poorly developed. At this time a tumor may take, establish itself, and possess sufficient growth potential to keep ahead of the developing immunological rejection forces. This would explain the failure of tumors to grow following excision and reimplantation in the same host, since at this latter reinoculation time the immunological forces of the host are more fully developed and cause rejection of the second graft. However, in these experiments when the original tumor was allowed to remain, metastasis occurred, throwing some doubt on the presence of immunological rejection forces at the time of metastasis.

Our results may be explained if the phenomenon of acquired tolerance is considered to be dependent on the presence of living foreign cells. In this circumstance the host allows growth of the tumor during a period when his immunological capacity is inadequate and the tumor by its growth maintains a state of acquired tolerance. Assuming metastasis to be new homotransplants, their occurrence may be evidence of persisting acquired tolerance due to the presence of the primary tumor. When the tumor is removed before the occurrence of metastasis, all living foreign tissue is removed, the animals are no longer tolerant, and therefore fail to accept retransplantation of the same tumor.

## SUMMARY

Tumor homografts will take and grow progressively between inbred strains of mice provided transplantation is performed within 3 hr after birth. These tumors retain their original histocompatibility characteristics despite growth in an homologous animal. It is proposed that living foreign tissue is required for the persistence of acquired tolerance.

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# THE EXPERIMENTAL DESIGN OF STUDIES TO EVALUATE THE CHEMOTHERAPY OF SOLID TUMORS AS DEMONSTRATED BY THE USE OF TRIETHYLENETHIOPHOSPHORAMIDE*

GEORGE E. MOORE, WILLIAM H. WEHR, TOMPKINS C. WATSON, AND ARTURO BELTRAN

In recent years there has been an ever increasing emphasis on the investigation of compounds with anti-tumor activity. The majority of clinical studies have been carried on in patients with leukemia and the malignant lymphomas.

A definite need exists for the systematic trial of these compounds in patients with solid tumors. Although the clinical effectiveness of most chemotherapeutic compounds upon solid tumors has been disappointing, tumor regression and suppression such as encountered in this study would indicate that future success will be obtained in this field. The importance of adding these new therapeutic techniques to our surgical armamentarium for the further control of malignancy is obvious.

A national program coordinating the exchange of information concerning synthesis of compounds, screening for anti-tumor activity, initial pharmacology studies, and clinical trials has been established. The Clinical Panel of the National Cancer Chemotherapy Center has made recommendations concerning the detection of compounds for human trial, criteria of evaluation, experimental design, and the number of patients which must be studied in order to obtain a valid evaluation of any one compound.

Selection of patients with measurable tumors is most important since after many discussions *the only acceptable criteria for chemotherapeutic effectiveness has been resolved to be the reduction in the size of the tumor by direct or indirect measurement.* Of course, demonstrable extension of life is the ultimate measure of chemotherapeutic effectiveness, but even patients with rapidly growing tumors such as glioblastoma or gastric, pancreatic, and lung carcinoma could hardly be evaluated in less than two years. Such ancillary criteria as changes in the histological appearance of the tumor, improvement in the biochemical status of the patient, weight changes, etc., are of interest in cataloging the pharmacologic effects of the drug but should never be used to measure anti-tumor activity.

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Changes in the leukemias are relatively easy to measure and therefore these diseases have been a popular means of evaluating chemotherapeutic agents However it is important to realize that a compound which may be effective against one tumor may have no effect against others and thus it is unfortunate that so many agents have been evaluated both experimentally and clinically only against the leukemias

It is obvious that even a large teaching hospital does not have sufficient numbers of patients with metastatic malignancy suitable for objectively evaluating many compounds except over a prolonged period of time This circumstance has forcefully indicated the need for establishing cooperative studies in which the data of several investigative groups are combined Five such groups have been formed or are being formed with the guidance of the Clinical Panel Each is comparing the effectiveness of 2 agents such as Triethylenethiophosphoramide (TSPA) versus nitrogen mustard (HN) or 6 Mercaptopurine versus 6 Chloropurine according to a single protocol developed to define the patients and tumors to be studied objective criteria of therapeutic effectiveness, and an acceptable method of randomizing the distribution of the patients in each group

Using this cooperative study technique a statistically valid comparison of 2 compounds should be accomplished in a period of months rather than years

To illustrate the difficulties and successes of the initial clinical trials of a chemotherapeutic drug which must be completed before a final evaluation by the cooperative study technique is rational our investigation of Triethylenethiophosphoramide (TSPA)* and Actinomycin D** follows Only the results of TSPA will be mentioned in detail since to date there has been no evidence of therapeutic effect of Actinomycin D against solid tumors in the adult

Triethylenethiophosphoramide was initially given to a series of advanced cases to establish the maximum tolerated dosage and as a consequence a course of 5 daily i v injections of 0 2 mg /kg was proposed for a definitive study of its anti tumor effects

In a brief study of the effects of a second course of therapy those patients who did not develop a hematologic depression and showed no tumor response after 4 wks were given a second course at the same dosage level or slightly higher Immediately 1 patient developed severe agranulocytosis and 2 others developed infections which they could not control In none of the patients given a second course of therapy were additional favorable tumor responses noted

Evaluation of the effectiveness of maintenance therapy was also attempted Briefly it was found that a weekly injection of 0 2 mg /kg intravenously was the maximum tolerated dosage and even then most patients had a continuous depression of the peripheral leukocyte count Again it was noted that no patient responded to this therapeutic regime who had not responded to the initial treatment

Following these preliminary investigations a comparative study was initiated employing Actinomycin D after completing similar pharmacologic

*TSPA furnished through the courtesy of Dr J M Ruegsegger of Lederle Laboratories

**Actinomycin D furnished through the courtesy of Dr Frederick K Heath of Lederle Laboratories

studies.† A protocol outlining the eligibility of patients, initial chemical and hematological information to be obtained, dosages and measurement techniques was completed, and a flow sheet for follow-up examinations printed. Tumor measurements were recorded weekly. The distribution of patients between the 2 agents was randomized by sealing in separate envelopes cards indicating the therapy to be employed. Random series were set up for each kind of malignancy to be studied.

## RESULTS

Since the purpose of this paper is to indicate aspects of planning chemotherapeutic investigations only, a short summary of the results obtained with TSPA will be given, and no effort will be made to review the negative results in patients given Actinomycin D.

Evaluation of tumor response has been separated into 4 simple classes as indicated in Table I. Class "O" includes patients dying within 3 wks. of treatment or given an incomplete course of therapy. Deaths have been classified as to whether the therapy was not a factor, an indirect factor, or a direct causative factor in a patient's death.

The only striking remissions seen in this study occurred in patients with breast cancer as represented by the following cases.

The wife of one of our staff members with extensive abdominal and liver metastases and rapidly accumulating ascites was unresponsive to ovariectomy and steroid therapy and was terminal before given TSPA. Three weeks later the liver rapidly reduced in size, alkaline phosphatase levels fell from 280 to 80 units, and the ascitic fluid did not recur. She was discharged from the hospital and has resumed many of her normal activities. To date this remission has lasted 3 mo. No further therapy has been given.

Three women with widespread metastases had remissions lasting about 2 mo. during which time almost all of the palpable lesions completely disappeared leaving only pigmented spots to indicate the tumor site. These areas were biopsied and some were found to contain either small clumps of viable tumor cells or bizarre "irradiation cells," whereas in others no tumor cells could be identified. Unfortunately after these brief remissions the nodules reappeared rapidly and additional metastases appeared. In 2 patients, a further remission of 2 mo. was obtained with retreatment but severe bone marrow depression prohibited further therapy. A fourth patient enjoyed a similar response of her soft tissue lesions, but simultaneously skeletal metastases were enlarging.

It is noteworthy that this study is not complete since too few patients have been seen to allow valid evaluation. The approximate number of patients necessary to evaluate a single compound given at a dosage level for malignancy of a single type has not been determined. Probably, it will be between 10 and 20 patients with each type of tumor—a formidable number for most clinics. This again emphasizes the need for cooperative studies.

## DISCUSSION

It is immediately apparent that these results differ from those reported by Bateman and co-workers.[1] However, careful study of Bateman's paper reveals that probably the majority of the excellent responses she docu-

†Preliminary data obtained from Dr. Sidney Farber.

*Table 1 Triethylenethiophosphoramide Therapy*

| DIAGNOSIS | CLASS O INCALCULABLE | CLASS 1 TUMOR CONTINUES TO INC SIZE | CLASS 2 TUMOR FAILS TO INC SIZE | CLASS 3 TUMOR DIMINISHES IN SIZE | CLASS 4 TUMOR DISAPPEARS COMPLETELY | DEATH NOT A FACTOR | DEATH INDIRECT FACTOR | DEATH DIRECT FACTOR | TOTAL IN SERIES |
|---|---|---|---|---|---|---|---|---|---|
| Breast | 3 | 2 | | [illegible] | | 3 | | 1 | 10 |
| Ovary | | 1 | | | | 1 | | | 1 |
| Lung | 3 | 1 | | | | 3 | | 1 | 4 |
| Prostate | | 1 | | | | | | | 1 |
| Oral Cavity | 1 | 6 | | | | [illegible] | 1 | 1 | 7 |
| Gastrointestinal | 9 | [illegible] | | | | | | 1 | 16 |
| Melanoma | | [illegible] | | | | 2 | | 1 | 3 |
| Miscellaneous | | 7 | | | | 4 | | | 7 |

mented were obtained by local injection rather than systemic administration. In contrast, the report of Shay and Sun[2] is compatible with the findings of the present study. They reported objective tumor response only in patients with breast and ovarian cancer.

The toxicity of TSPA should not be minimized. Shay reported that the drug probably contributed to the deaths of 3 patients in his series of 17 patients through severe bone marrow depression. In this study, a relatively high dosage over a short period of time was chosen deliberately to obtain the maximal tumor response. Three patients died as a direct result of bone marrow depression and terminal infection, and 2 others with thrombocytopenia died from hemorrhage from fungating tumor masses.

In this group of 19 patients, 15 had a depression of their peripheral white cell count below 3,000 and 13 of these were below 1,000. In 12 patients the platelet count was depressed below 100,000 and in 6 below 50,000. Maximal depression of peripheral leukocytes occurred 2 wks. following the last injection (range 5 to 27 days, median 15 days) and platelets 4 to 28 days (median 17 days).

Actinomycin was given intravenously in daily increments to a total of 75 gamma/kg. over a 5 day period. Subsequently, weekly injections of 15 gamma/kg. were given.

No striking tumor responses were seen nor were there any fatalities attributable to this drug. Thrombocytopenia regularly occurred soon after the therapy was completed, and then returned to normal levels in a few days. Leukopenia was clinically insignificant.

## CONCLUSIONS

1. Many new potential cancer chemotherapeutic chemical agents requiring clinical evaluation can be expected in the next few years. There is a great need for clinical investigators to engage in preliminary human pharmacologic studies and formal attempts to evaluate the anti-tumor activity of these compounds.

2. The only practical method of objectively evaluating chemotherapeutic agents as to anti-tumor activity in a reasonably short time involves setting up cooperative studies between clinical groups in order to pool available patients with various tumor types, utilize a common protocol so as to standardize therapy, and establish rigid statistical controls.

3. The steps preliminary to acceptance of a final protocol suitable for a cooperative study are illustrated by our preliminary investigations with Triethylenethiophosphoramide.

4. Brief mention is made of the clinical results of a comparative study of TSPA and Actinomycin D. Several spectacular, brief remissions were obtained in patients with metastatic breast cancer given TSPA intravenously.

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# THE EFFECT OF CANCER INHIBITOR DRUGS ON THE TAKE OF WALKER CARCINOSARCOMA 256 IN RATS*

GERALD O. MCDONALD, ERNESTO P. CRUZ, AND W. H. COLE

Metastases from primary tumors develop in numerous ways including primarily lymphatics, implantation and venous emboli. The danger of implantation of cancer cells into the suture line of the colon was first emphasized by Morgan and Lloyd Davies.[1] Perhaps of more importance to the patient (*in intestinal cancer*) *is hepatic metastases which develop from* venous emboli. One of us with associates[2] has demonstrated cancer cells in venous blood and saline perfused through the vessels supplying a carcinoma of the rectum by operation, and has emphasized the necessity of ligating the vascular trunks prior to resecting and even manipulating tumors of the colon and rectum. Fisher and Turnbull[3] have carried the investigation further and report demonstration of cancer cells in the venous blood in 32 per cent of 25 carcinomas of the colon removed at surgery. We have concluded that in spite of care and caution in manipulating these tumors, numerous emboli of tumor cells are discharged into the veins draining the tumor site. These cells reach the liver, some of them develop vascular attachments and form hepatic metastases. We have postulated that cells desquamated into the wound, as well as venous emboli, might be killed if treated by anticancer agents before they develop vascular attachments. Perhaps of more importance, the microscopic metastases held in check by the patient's immunity might be killed or prevented indefinitely from growing if anticancer agents are given frequently after the tumor is excised.

This study was undertaken to determine if cancer inhibitor drugs would prevent or affect the hepatic growth of tumor cells artificially implanted into the portal circulation of susceptible animals (rats). These tumor cells would closely simulate those discharged into the portal system at the time of surgical resection of a colon carcinoma.

## METHOD

The Walker rat carcinosarcoma 256 was chosen because of its hardy characteristics and its ability to grow readily when transplanted. This tumor was maintained by subcutaneous transplants in female Sprague Dawley rats weighing between 125 and 175 gm. All work was performed under aseptic operating room conditions.

The tumor cell suspension was prepared by first finely mincing the tumor and then adding a small amount of saline as recommended by Locke and associates[4] for producing hepatic metastases with the $V_2$ tumor in rabbits. This mixture was filtered through a fine stainless steel mesh producing a filtrate consisting almost entirely of single cells. A quantitative cell count was then obtained and the cell suspension diluted with physiologic saline so that 1 cc. of the suspension contained between 110,000 to 125,000 cells.

Under Pentobarbital anesthesia the peritoneal cavity of the rat was opened

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and a large mesenteric vein isolated One cubic centimeter of the cell suspension was then injected into the isolated vein Hemorrhage following withdrawal of the needle from the vein was controlled with gentle pressure Groups of 10 to 20 animals were operated at one time so the tumor cell injections could be accomplished as rapidly as possible The animals receiving injections of the inhibitor drugs received the tumor cell injections before the control groups This was done to circumvent the possibility that if there was a decrease in the virulence of the cells after standing a longer period of time (30 to 60 min) the more virulent cells would be received by the treated group

Three anticancer agents were used—azaserine nitrogen mustard and thiotepa In the first group of animals azaserine (2 0 mg /kg of body weight) was injected into the portal circulation 1 min after the injection of the tumor cells In the second group nitrogen mustard (0 5 mg /kg of body weight) was injected in the same manner In the third group nitrogen mustard was injected into a peripheral (foot) vein 1 hr following the tumor cell injection In the fourth group nitrogen mustard was injected into the peritoneal cavity 1 hr following the intraportal tumor cell injection In the fifth and sixth groups thiotepa (2 0 mg /kg of body weight) was injected by the intraportal and intraperitoneal routes respectively, following the intraportal injection of a double strength tumor cell suspension containing 220 000 to 250 000 cells

In each small group of 10 to 20 rats operated at the same time half of the animals always served as controls We felt this method eliminated as many variables as possible the most significant variable being the different tumors used in preparing the suspension for each procedure

RESULTS

The results obtained with azaserine were not favorable following the intraportal injection of 2 0 mg /kg of body weight 1 min after the tumor cell injection In 42 treated and 14 control animals the incidence of tumor growth in the liver was about the same for both groups being 95 2 per cent and 88 6 per cent respectively

As reported previously[9] when nitrogen mustard was given in an effort to kill the injected cell results were definitely encouraging In a control group of 38 rats the percentage take was 97 4 per cent whereas in 36 rats treated by injection of 0 5 mg nitrogen mustard into the portal vein 1 min after injection of cells the percentage take was 19 4 per cent

In another experiment in which nitrogen mustard was injected into the peritoneal cavity and still another in which the drug was injected into a systemic vein (in each case 1 hr after injection of cells) the results were almost as favorable although the groups were smaller

In Table 1 are listed the results of injection of 1 dose of thiotepa into the portal vein 1 min after the injection of the cancer cells In this experiment the number of cells was increased to 225 000 doubling the number injected in the previous experiments With the increased number of cells there was 100 per cent take in the 23 control animals In the 27 treated animals the percentage of takes was 67 per cent

Table 2 lists the results of 1 injection of thiotepa into the peritoneal cavity 1 hr following injection of the cancer cells in the portal vein The

*Table 1 Effect of One Dose (2 0 Mg/Kg of Body Weight) of Thiotepa Injected Into the Portal Vein of Rats One Minute After Injection of the Cancer Cells*

| GROUP | NO RAT | PER CENT TAKE |
|---|---|---|
| Treated | 27 | 67 0% |
| Control | 23 | 100 0% |

*Table 2 Effect of One Dose (2 0 Mg/Kg of Body Weight) of Thiotepa Injected Intraperitoneally One Hour After Injection of Cancer Cells Into the Portal Vein*

| GROUP | NO RAT | PER CENT TAKE |
|---|---|---|
| Treated | 30 | 14 3% |
| Control | 30 | 75 9% |

percentage of takes was 75 9 per cent in 30 control animals and 14 3 per cent in 30 treated animals

DISCUSSION

Prior to obtaining the results recorded herein it was necessary for us to demonstrate the feasibility of injecting 1 cc of solution into the relatively small mesenteric vein of the rat without complications of spillage and hemorrhage Following this we had to determine if the Walker 256 tumor would grow in the liver following portal vein injection of a tumor cell suspension Lucke and Breedis[4] had shown the liver to be a favorable site of growth of the rabbit $V_2$ carcinoma when a cell suspension of this tumor was injected into the portal vein Using their method in preparing the cell suspension modifying it to obtain a suspension of single cells we were able to show a high percentage of takes in the liver following the intraportal injection of a suspension containing 100 000 to 150 000 cells There were some deaths from anesthesia and hemorrhage These animals were discarded from our series

Admittedly these series are not large yet the results appear to be consistent in all groups The control rats tended to die by the 21st postoperative day and their livers contained multiple tumor nodules In comparison those treated rats which did develop tumor had fewer and much smaller tumors than did the control animals However from our results we wished primarily to learn the effectiveness of the inhibitors in completely preventing not just decreasing hepatic growth

We realize the difficulty in transferring the results of animal experimental work to human clinical procedures However the results of this experimental animal work have been sufficiently encouraging to stimulate us to give nitrogen mustard to patients having resection of tumors which metastasize by vein as well as by lymphatics We have been giving a 4 day course of nitrogen mustard the first dose of 0 1 mg/kg of body weight being given the day of operation in intestinal tumors we have given the first dose into the peritoneal cavity To date our maximum dose to freshly

operated patients has been 6.0 mg; at present we are limiting the therapy to patients under 71 yr of age.

### SUMMARY

Results of prophylactic anticancer therapy are reported herein. The experimental method of injecting a tumor cell suspension into the portal vein of rats was chosen because it resembled the mechanism of production of hepatic metastases in carcinoma of the digestive tract. When nitrogen mustard was injected *into the portal vein, into the peritoneal cavity* or into a peripheral vein, it was effective in decreasing the incidence of "take" of the Walker carcinosarcoma cells following their portal vein injection in the rat. Thiotepa was also effective in decreasing the incidence of tumor "takes" in the liver when injected intraperitoneally, whereas it was only moderately effective when injected directly into the portal vein. All series are small and are being enlarged. Azaserine when similarly injected into the portal vein was not effective in preventing tumor growth in the liver.

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## HORMONAL FACTORS AFFECTING GROWTH OF EXPERIMENTAL SECONDARY BONE TUMORS*

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The investigations during the past 10 yr have shown to an increasing extent the significance of various systemic factors which affect the process of malignancy. The promoting and inhibitory action of hormonal factors is evident in many clinical and experimental tumors. In this experiment it was attempted to evaluate the effects of estrogens, androgens and cortisone preparations commonly used in the treatment of metastatic bone disease

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on the intraosseous transplants of Walker Carcinoma 256 a highly ana plastic mammary carcinoma of rats

METHOD

Hooded male rats of the Royal Victoria Hospital strain weighing 120 to 150 gm were used Male animals were selected because of metabolic varia tions in the females caused by sexual cycle The Walker Carcinoma 256 was transplanted from Sprague Dawley strain to our colony using a stand ard intramuscular transplantation for comparison with the growth of the tumor in intraosseous implants

Intraosseous implantation was performed using the following method The animal was anesthetized with nembutal and a transverse skin incision of 1 cm length was made on the anterior surface of the left knee joint The extensor tendon and the anterior capsule of the knee joint were tran sected and the lower end of the femur exposed Using a dental burr No 7 a hole was drilled in the lower end of femur parallel to the shaft and about 3 to 5 mm deep A tumor implant weighing 10 mg was placed in the opening Subsequently the burr hole was occluded with a stainless steel screw The extensor tendon and skin were resutured Animals tolerated the procedure well and no operative mortality was encountered

Intraosseous implantation was performed in 144 rats and the animals were divided into 4 equal groups Group 1 (Control) No hormones ad ministered Group 2 (Cortisone) Intramuscular administration of Corto-gen' (Schering) 5 mg /day (0 05 mg /gm of body weight) from the day of implantation Group 3 (Estrogens) Intramuscular administration of Premarin (Ayerst) 2 mg /day (0 016 mg /gm of body weight) from the day of implantation Group 4 (Androgens) Intramuscular administration of Oreton (Schering) 5 mg /day (0 05 mg /gm of body weight) from the day of implantation

Twelve rats from each group were sacrificed each week Postmortem ex amination and a search for metastases in other organs was carried out in all animals The femur containing the tumor implant was fixed in 10 per cent formalin solution decalcified in Sequestrin $Na_4$ and sectioned longitudinally at $5\mu$ in thickness

RESULTS

A take was considered positive if any evidence of malignant tissues was found in the section The invasiveness of the tumor implant was judged by the destruction of the adjacent bony tissues The resistance of various layers of bone to tumor implant was studied The epiphyseal cartilaginous layer of the lower end of the femur appeared to be a significant barrier to the growth of the tumor but once the tumor implant crossed the carti lage zone it spread relatively easily into the marrow cavity and destroyed the trabeculae of spongy bone and marrow cells The compact bony layer was more resistant to tumor invasion than the spongy layer and marrow cavity

1 The *Control Group* showed 77 7 per cent takes of the implants of the Walker tumor with moderate extension of the tumor and moderate re active bone formation

2 The *Cortisone Group* showed a 94 4 per cent take of the implants



Fig 1 (Control) Walker carcinoma 256 growing in the marrow cavity x400 H & E



Fig 2 (Cortisone) Necrosis in Walker carcinoma growing in the marrow cavity x400 H & E



Fig 3 (Estrogen) Walker carcinoma invading marrow tissue x400 H & E



Fig 4 (Androgen) Fibrosis of the marrow cavity around invading Walker carcinoma x400 H & E

At 7 days the implant showed more extension than the controls but at 14 days the degenerative changes and necrosis were observed and at 21 days necrosis was more frequently observed and more extensive than in any other group The formation of reactive bone was not affected by the cortisone in the dosage used in this experiment

3 The *Estrogen Group* had 88 9 per cent takes of the implants Enhancement of the invasiveness of the tumor implants was observed in 25 out of 36 rats Reactive bone formation was slower than the control group

4 The *Androgens Group* showed the lowest 'take (55 5 per cent) The extension of the tumor was considerably less than in the controls A zone of *fibroblastic and osteoblastic proliferation was observed around the implants* in the majority of animals in this group This group showed more reactive bone formation than any others

DISCUSSION

**Effects of Hormones on Secondary Tumor Growth in Bone Tissue.** The bone tissue and the transplanted tumor tissue may be affected in a different manner by the same hormonal stimulant This fact probably explains the effects of hormones used in this experiment on the growth of intraosseous transplants of Walker Carcinoma 256 The final result depends on the various inhibitory and promoting effects the local resistance to tumor growth and the malignant potentialities of transplanted tumor tissue[1]

The estrogen treated animals showed less bone formation than the controls This might be partially responsible for the enhancement of tumor growth observed in this group That mammary carcinoma can be induced experimentally in rats by administration of estrogens and that tumor growth can be enhanced by estrogen administration[2] suggests that estrogens may be the most important dependency factor in mammary cancer However as estrogens have different effects on bone in various species the data should not be extrapolated to other species[3]

The effect of cortisone on bone is probably non specific and secondary to other systemic effects Even in high doses it does not affect bone metabolism except indirectly by loss of protein nitrogen from the body In this experiment the highest take of intraosseous implants was observed in the cortisone treated group The tumor had also more tendency to spread during the first and second week It is possible that cortisone promoted the tumor growth during the period perhaps by suppressing the antigenic reaction The necrosis and regression of tumor transplants seen at later periods might be due to the suppressive effect of cortisone on pituitary hormone production which in turn reduces estrogen production This effect of cortisone on intraosseous implants differs from that on subcutaneous transplants which exhibited decreased growth rate from the beginning[4]

Androgens have a promoting effect on osteogenesis It is likely that increased bone growth increased the resistance to the invasion of the implanted Walker Carcinoma and might have been responsible for regressive changes and decreased growth rate observed in the tumor implants in this experiment On this basis androgens might be expected to produce objective improvements in patients with bone secondaries from breast carcinoma However the possibility that androgens neutralize the effects of endogenous estrogens should also be considered It may be relevant that Bethune observed that in patients with breast cancer the prognosis after hypophysectomy was better in cases with bony metastases than in those with involvement of the soft parts

**Tumor Growth in Bone Tissue.** The study of the effects of hormones on intraosseous implants of Walker Carcinoma 256 permits also certain conclusions as to the tumor growth in general This experiment demonstrates that the secondary tumor growth is more restricted in osseous tissue than in muscle tissue On intramuscular transplantation the tumor grows to a palpable size after 1 wk and by the third week has enlarged sufficiently to produce cachexia and death of the host The takes are almost 100 per cent On the intraosseous implantation the tumor does not extend more than 1 to 1 5 cm inside the marrow cavity during a corresponding period of time This inhibition of the expansion of the tumor appears to be related to

resistance of bony tissue to invasion due to some local inhibitory factors

The resistance of various components of bony tissue to tumor invasion varies As observed in this experiment the growing epiphyseal cartilage appears to be most resistant to invasion by malignant cells and the compact bone to be considerably more resistant than spongy bone The resulting growth pattern of the tumor therefore differs from that in soft issues

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# COMBINED THERAPY (BILATERAL ADRENALECTOMY AND RADIOACTIVE PHOSPHORUS) FOR CARCINOMA OF THE BREAST WITH WIDESPREAD METASTASES TO BONE*

Harvey Krieger William E Abbott William D Holden
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In 1952 Huggins[1] demonstrated that bilateral oophorectomy and adrenalectomy were effective forms of palliative treatment for patients with widespread metastases from carcinoma of the breast It has now become apparent from the various reports in the literature that only 40 per cent of the patients have tumors which are estrogen dependent and therefore derive significant benefit from the aforementioned procedures[2,3] Although various criteria and methods for the selection of patients have been recommended it is impossible in any individual case to predict the magnitude and duration of palliation When there are extensive and widespread metastases to bone it is not possible to treat all the various sites by external irradiation However the availability of radioactive isotopes offers an opportunity for internal irradiation Friedell and Storaasli[4] in 1950 reported a series of 12 patients with widespread metastases to bone who were treated with radioactive phosphorus ($P^{32}$) This isotope has a physical half life of 14 3 days and emits a beta particle which has an average range of 2 mm Therefore the radiation is confined primarily to the tissue in which it is deposited Since bone con

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DISCUSSION

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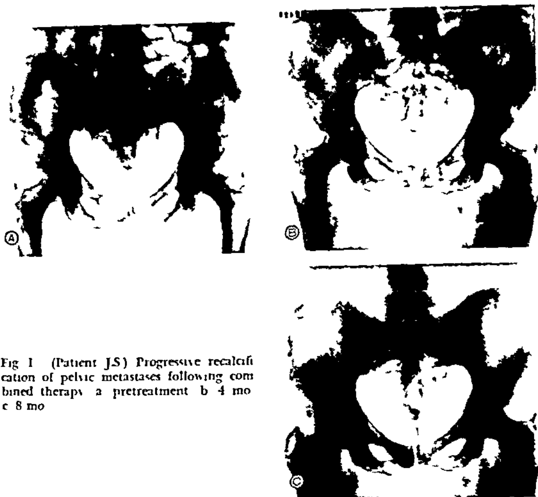



Fig 1 (Patient J.S) Progressive recalcification of pelvic metastases following combined therapy a pretreatment b 4 mo c 8 mo

the combined therapy and has been able to carry on her occupation as a school teacher as well as participate in such activities as gardening hiking and swimming Two of the patients (J.S and L.Pr) had no evidence of visceral metastases at the time of operation They are asymptomatic 9 and 29 mo respectively after therapy The other 3 patients (V.G M.Mc and L.Pe) all had visceral metastases at the time of operation V.G and M.Mc died 18 and 11 mo after therapy although they both had objective improvement in their osseous metastases and led normal active lives until shortly before death L.Pe is living 10 mo after therapy but now has ascites and her liver function tests show abnormalities which are compatible with hepatic metastases It is apparent that the prognosis is much better in those patients who at the time of therapy have their metastases confined to the skeletal system

The total amount of radioactive phosphorus given to each of the 5 patients was comparable to that employed by Friedell and Storaasli [4] Purpura occurred as a complication in 9 of their 12 patients The platelet count fell to 50 000 or less in 6 of their patients and a depression of leukocytes to 3 000 or less occurred in 5 patients Purpura has not occurred in any of the 5 patients treated with $P^{32}$ after adrenalectomy nor was there any significant prolonged depression of the platelet or leukocyte counts Table 1 lists the hemogram for each of the 5 patients before they received $P^{32}$ as well as the maximum change in the hemogram following the full course of therapy The

tains more phosphorus than any other tissue, the major fraction of any administered $P^{32}$ will accumulate in the skeletal system This has been demonstrated both experimentally and in tissues obtained at autopsy from a patient treated with $P^{32}$ [4] Seven of the 12 patients who were treated with $P^{32}$ had significant relief from bone pain and weakness Two patients had significant objective improvement in their osseous lesions demonstrable by recalcification as shown by serial roentgenograms The only complication of importance which occurred with this form of therapy was severe depression of the hematopoietic system

When several patients who had previously been adrenalectomized were given comparable amounts of $P^{32}$, it was noted that there was no significant depression of the hematopoietic system Therefore it was decided to determine whether there was any significant difference in the uptake distribution and excretion of $P^{32}$ before and after adrenalectomy

## METHOD

Six patients with widespread osseous metastases who were to undergo adrenalectomy and oophorectomy were admitted to the Metabolic Division of the University Hospitals of Cleveland for this study Three days before adrenalectomy a tracer dose of radioactive phosphorus (0 5 to 1 0 mc) was administered intravenously All urine and feces were collected in 24 hr periods up to the morning of operation At the time of adrenalectomy a segment of rib containing both metastatic tumor tissue and normal bone was obtained for assay Two to 4 wks after adrenalectomy a second tracer of $P^{32}$ was administered and again urine and feces were collected for 3 days and on the fourth day a segment of rib similar to that removed at the time of adrenalectomy was obtained

**Isotope assay methods** *Urine* Three 1 ml aliquots of each 24 hr urine collection were pipetted into metal planchets dried and counted with a Geiger Muller counter *Feces* Each 24 hr collection of feces was brought to a known volume with distilled water wet ashed with concentrated nitric acid, and 1 ml aliquots placed in planchets dried and counted *Bone* Bone samples were wet ashed with nitric acid weighed and counted in the same manner as the urine and feces

After the second tracer study was completed the patients were given a therapeutic course of $P^{32}$ This consisted of 2 mc weekly until a total dosage of 9 to 18 mc was given Complete blood studies including hemoglobin red blood cell white blood cell and platelet counts were obtained prior to the treatment with $P^{32}$ and periodically thereafter

## RESULTS AND DISCUSSION

Five of the 6 patients included in this study were given a therapeutic course of $P^{32}$ after adrenalectomy These 5 patients improved subjectively as manifested by diminution or complete disappearance of bone pain and increase in strength and appetite Four patients who had been bedridden before adrenalectomy were subsequently able to carry on all of their normal activities Objective improvement in all 5 of these patients was evident by progressive recalcification of osseous lesions as shown by serial roentgenograms Figure 1 shows the progressive recalcification of the pelvic metastases in patient J S over an 8 mo period This patient had striking benefit from

*Table 2 Tissue Uptake of $P^{32}$ Before and After Adrenalectomy*

| PT | TYPE OF TISSUE | PRE ADRENALECTOMY | POST ADRENALECTOMY |
|---|---|---|---|
| | | COUNTS/MIN/CM | |
| M M | Normal Bone | 3 461 | 5 313 |
| | Tumor Adjacent Bone | 9 713 | 10 316 |
| | Tumor Tissue | 22 606 | 25 153 |
| J S | Normal Bone | 26 736 | 19 519 |
| | Tumor Adjacent Bone | 50 753 | 36 861 |
| | Tumor Tissue | 39 589 | 96,237 |

tissue were assayed normal bone bone adjacent to metastatic tumor and tumor tissue It should be noted that in both patients pre and postadrenalectomy the uptakes of $P^{32}$ in tumor tissue and bone adjacent to tumor was significantly greater than the uptake by normal bone This agrees with the findings of Friedell and Storaasli[4] Following adrenalectomy in patient J S the ratio of $P^{32}$ uptake in tumor tissue as compared to the uptake in normal bone was significantly increased above the ratio of uptake in these tissues before operation

SUMMARY

1 Five patients with widespread metastases to bone from carcinoma of breast were given a therapeutic course of P following bilateral oophorectomy and adrenalectomy

2 All 5 patients were significantly improved both subjectively and objectively with respect to their bone metastases and none had any clinically significant depression of the hematopoietic system

3 Although to date we have been unable to demonstrate conclusively a significant difference in the distribution of $P^{32}$ before and after adrenalectomy we believe that the administration of a course of $P^{32}$ after adrenalectomy provides a maximal form of palliation for the patient with widespread metastases to bone from carcinoma of breast

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*Table 1 Hemograms Before and After Treatment for 5 Patients Who Received Therapeutic Course of $P^{32}$ After Adrenalectomy*

| PATIENT | AGE | $P^{32}$ DOSAGE | | Hgb GRAMS | HEMOGRAM RBC x $10^6$ | WBC | PLATELETS |
|---|---|---|---|---|---|---|---|
| V G | 40 | 16 mc in 67 days | I** | 14 1 | 4 63 | 4 400 | |
| | | | M* | 10 6 | 3.53 | 2 150 | 145 080 |
| L Ir | 69 | 15 mc in 37 days | I | 13 7 | 5 33 | 5 300 | 400 000 |
| | | | M | 12 8 | 4 30 | 2 950 | 135 000 |
| M Mc | 52 | 9 2 mc in 31 days | I | 9 0 | 3 17 | 4 850 | 128 990 |
| | | | M | 8 9 | 3 17 | 3 050 | 66 880 |
| L Le | 36 | 16 mc in 53 days | I | 11 9 | 4 00 | 9,500 | 545 160 |
| | | | M | 11 1 | 3 86 | 5 850 | 265,230 |
| J S | 50 | 18 mc in 36 days | I | 14.5 | 4 70 | 5 600 | 400 900 |
| | | | M | 12.5 | 4 14 | 4 300 | 66 240 |
| Average | 49 | 14 8 mc in 44 8 days | I | | 4 36 | 5 930 | 368 762 |
| | | | M | | 3 80 | 3 660 | 135 486 |
| Average (Friedell & Storaasli) | 48 | 16 0 mc in 41 5 days | I | | 3 88 | 8 800 | 307 000 |
| | | | M | | 3 15 | 2 000 | 90 000 |

**I retreatment Hemogram
* Maximal change after $P^{32}$ treatment

average values for the 12 patients treated by Friedell and Storaasli are also shown in Table I The difference in the magnitude of the depression of the hematopoietic system in these 2 groups of patients suggested the possibility of some change in the uptake excretion or distribution of $P^{32}$ following adrenalectomy

Two of the patients (J S and L Pe ) showed hypercalcemia and negative calcium balance (600 to 800 mg daily urinary calcium with an intake of 300 mg ) The calcium balance of both of these patients became positive and their serum calcium concentrations were restored to normal following adrenalectomy

It was thought that if the rapid and progressive bone destruction were reduced or eliminated following adrenalectomy increased amounts of radio active phosphorus might be deposited in metastases in association with the recalcification of these lesions In order to investigate these possibilities $P^{32}$ excretion and tissue uptake studies were undertaken

The excretion of $P^{32}$ in the urine and feces over a 3 day period was essentially the same preoperatively and 2 to 4 wks postoperatively indicating no significant change in the retention of $P^{32}$ after adrenalectomy in this group of 5 patients

An attempt was made to determine whether there was a more selective uptake of $P^{32}$ by tumor tissue as compared to normal bone after adrenalectomy Satisfactory rib segments were obtained for assay from only 2 of the patients before and after adrenalectomy Table 2 shows the uptake in these rib segments expressed as counts/min /gm of wet tissue Three types of

appraisal of their internal mammary lymph nodes, 1 had carcinoma involving axillary and one or more internal mammary lymph nodes and 3 had involvement of axillary lymph nodes only. No patient had carcinomatous deposits in the internal mammary lymph nodes alone.

## DISCUSSION

**Distribution of Radiogold in the Absence of Demonstrable Metastatic Neoplasm.** Regardless of the site of placement of the radiogold, there was usually a generalized distribution in identifiable lymph nodes. Of the 332 lymph nodes examined in these 20 patients, only 60 were not found to contain any radiogold.*

Although in some individuals the lymph nodes nearest the injection site contained the greatest concentration of radioactive particles, there were many instances in which the highest node in the axilla or one of the nodes along the lateral portion of the axillary vein or in the region of the subscapular vessels had an equally high count. Considerable variation in counts per node was quite common and this could occasionally but not always be correlated with the size of the lymph node. Lymphatic flow to the internal mammary lymph nodes was merely a trickle as compared with that to the axillary chain. Although a comparison of the gold deposited in axillary and internal mammary lymph nodes is most gross, the internal mammary lymph nodes contained less than 1 per cent to 2 per cent of the total radiogold deposited.* Even if it is assumed that the maximum number of internal mammary nodes were present and that the lymph nodes examined were representative, these figures would not be appreciably altered. It was quite clear that injection in any quadrant could be followed by the deposition of gold in the internal mammary chain. This distribution pattern as well as estimates of quantitative flow are similar to the observations of Hultborn.[2]

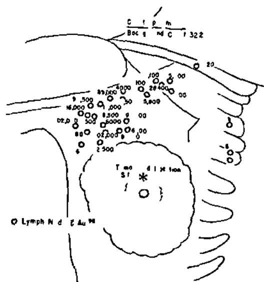


Fig 1. Location and activity of 27 lymph nodes removed from a radical mastectomy specimen in a patient receiving 1.64 mc of radiogold 24 hrs before surgery. Note the variation in activity of the various nodes. This could not be specifically correlated with size of the lymph node. In this instance the counts in the internal mammary nodes removed represent approximately 0.2% of total lymph node activity. None of the lymph nodes contained carcinoma.

* The criterion for deposition of radiogold in a lymph node was a count of at least twice background.

* There was one exception to this observation in that a patient with a medially situated lesion had 19 lymph nodes examined, 2 of which were internal mammary nodes and contained 15 per cent of the radiogold deposited.

# PROGRESSION OF LYMPHATIC METASTASES IN CARCINOMA OF THE BREAST*

COLIN G THOMAS JR

The surgical treatment of carcinoma of the breast has been based upon the premise that this neoplasm spreads primarily by the lymphatic route—a centrifugal embolic spread with progressive chain like involvement of more lymph nodes This concept plus the clinical evidence that there may be a segmental type of lymphatic flow from certain areas of the breast, has led to the extension of surgery beyond axillary node dissection in an effort to include a greater area of potential lymph drainage The findings of carcinomatous lymph nodes in these areas of extension has been considered justification for this approach The validity of these concepts however has never been carefully scrutinized experimentally since until recently most of the views on lymphatic drainage of the breast were based upon studies in the cadaver and the location of metastatic cancer These methods have not permitted a quantitative study of lymphatic flow and it has not been possible to appraise the relative importance of the various routes or under what conditions the accessory pathways may assume greater import Furthermore relatively little attention has been given to the alteration in lymph dynamics that occurs in the presence of metastatic neoplasm The purpose of this investigation was to make a quantitative study of lymphatic flow from various quadrants of the breast and in particular to appraise any significant alteration in lymph dynamics by the presence of lymph node metastases Radioactive colloidal gold which has been demonstrated to follow lymphatic pathways and to become phagocytized by the reticuloendothelial cells was selected for this study [1]

## METHOD

Tracer amounts of radiogold (0 1 to 1 0 mc) in a volume of 1 ml were injected into large tumors or adjacent smaller tumors in patients undergoing radical mastectomy In most instances injection was performed the day preceding surgery although in a few cases the time interval was as short as 1 hr Evaluation of the first group of patients did not include examination of the internal mammary lymph nodes More recently however one or more internal mammary lymph nodes has been removed from the second, third or fourth intercostal space as a diagnostic aid Examination of the surgical specimen was carried out within 24 hr all grossly identifiable lymph nodes being removed by sharp dissection In addition to determining their radioactivity the location of all nodes was charted and histologic examination was made for relative amounts of lymphoid elements and neoplasm

Thirty three patients with breast cancer were studied by these methods Twenty patients had no evidence of lymph node metastases and these included 8 with internal mammary node dissections Thirteen patients including 7 with internal mammary node dissections were found to have 1 or more metastatic lymph nodes containing cancer Of the 13 patients having an

*From the Department of Surgery School of Medicine University of North Carolina Chapel Hill N C Supported by a research grant (USPHS C 1915) from the National Cancer Institute of the National Institutes of Health Public Health Service

tion in lymphatic flow such as to the opposite breast diaphragm or hepatic lymphatics In these individuals there may be no demonstrable gold in axillary or internal mammary lymph nodes even though they contain lymphatic tissue as well as carcinoma

From these observations in patients with carcinomatous nodes it is evident that considerable alteration in normal routes of lymphatic flow is common as reflected by a) haphazard distribution of radiogold deposition b) the lack of radiogold in lymph nodes containing lymphatic tissue as well as cancer and c) a demonstration of radiogold in sites other than axillary and internal mammary lymphatics The most significant of these alternate pathways remains to be determined

## CONCLUSIONS

If the dissemination of radiogold can be used as a guide to lymphatic flow from the breast it is evident that particulate matter in any segment of the breast may pass through any of the lymphatics known to drain breast parenchyma Despite the clinical evidence that suggests some segmental lymphatic drainage from the breast [4-6] the predominant lymphatic flow from all areas as herein studied is toward axillary lymph nodes Less than 1 to 2 per cent of lymphatic flow is in the direction of the internal mammary lymphatics It is likely that other accessory pathways have similar import

Conditions are quite different and somewhat unpredictable once obstruction of some of the afferent lymphatic pathways occurs Where axillary lymph nodes are extensively involved by cancer internal mammary inframammary and all accessory routes of lymphatic flow are utilized In the presence of axillary lymph node metastases it is easy to understand the high incidence of internal mammary metastases It should be emphasized that whereas the axilla probably remains a more effective barrier against lymphatic spread of carcinoma because of what constitutes more or less multiple lines of defense the internal mammary lymph nodes are few small and probably provide a most ineffective barrier against carcinomatous spread

These studies would seem to have the following clinical significance In the absence of lymphatic metastases to axillary lymph nodes the inclusion of the internal mammary lymphatic chain in an effort to encompass the regional lymph flow from the breast enhances this possibility only by the percentage of lymphatic flow to this area This probably represents less than 2 per cent of total lymphatic flow It is likely but has not been substantiated experimentally that the quantitative flow to this area from medial portions of the breast may be somewhat higher

In the presence of axillary lymph node metastases particularly when they occur to such an extent that there is an obstruction in afferent lymphatics and alteration in lymphatic flow the internal mammary lymphatic chain represents only one of the alternate routes available—many of which are unpredictable Extirpation of the internal mammary chain under these circumstances would seem to provide a relatively insignificant removal of potential areas of lymphatic drainage

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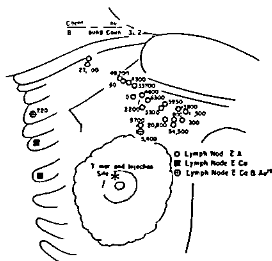


Fig 2 Location and activity of 23 lymph nodes removed from the surgical specimen of a patient with a neoplasm located immediately superior and slightly medial to the nipple All 3 internal mammary lymph nodes contained carcinoma with two completely replaced by neoplasm and the third nearly so The involved axillary node still retained considerable phagocytic function despite partial replacement by cancer

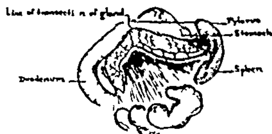
A contributing factor to the relatively minor roles played by the internal mammary lymphatics may be the small number as well as the small size of the lymph nodes present This would in effect produce a more ineffectual barrier and one that is soon replaced by neoplasm

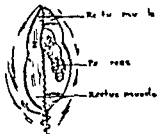
From these studies in patients without metastases and employing radiogold as a criterion of lymphatic flow it is evident that there is very little *segmental* lymphatic flow from quadrants of the breast All sectors drain primarily by way of the axillary lymph nodes but with some drainage to internal mammary nodes There was a suggestion that the internal mammary nodes are perhaps relatively more important in receiving afferent lymphatics from the medial portions of the breast However even here lymphatic flow is primarily in the direction of the axilla

**Lymphatic Drainage as Altered by the Presence of Carcinomatous Lymph Nodes** In evaluating the location of lymph nodes as well as the radiogold in patients with one or more lymphatic metastases several findings were of interest The presence of carcinoma in the internal mammary lymphatics was always associated with axillary metastases The location of the axillary metastases did not reflect the location of the primary tumor although in general the nodes of Rotter were most frequently involved with lateral quadrant lesions In several instances what would be considered the nodes nearest the tumor were uninvolved whereas only the apical axillary nodes contained carcinoma This skipping of lymph nodes has also been noted by Monroe[3] The presence of carcinoma was associated with a haphazard distribution of radiogold through the uninvolved lymph nodes as compared with the distribution in patients without metastases This irregular distribution of radiogold in uninvolved lymph nodes can best be explained on the basis of lymphatic blockage There are not a sufficient number of observations to be able to indicate a quantitative relationship between the number of lymph node metastases and alteration in lymphatic flow It is somewhat difficult to reconcile this observation with the fact that in many individuals all axillary lymph nodes are involved with carcinoma unless this involvement represents a retrograde permeation rather than embolic extension

The presence of more extensive axillary lymph node involvement by neoplasm but without any demonstrable lymphedema results in marked altera

Fig 1



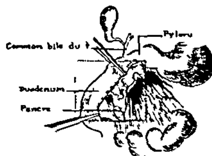




STAGE I

Division of the left limb of the pancreas

Elevation of the left limb to the rectus compartment with blood supply intact






STAGE II

Division of the blood supply to the transplant from below the peritoneum

Excision of the remaining pancreas and adjacent segment of the duodenum

islet cells and then observing whether the tissue would survive autotransplantation

METHOD

**Stage I (see Fig 1)** A left paramedian incision was made under intravenous nembutal anesthesia The rectus sheath was opened widely and approximately 7 cm of the rectus muscle excised The upper and lower borders of the rectus compartment were then closed thus creating a fascial envelope into which the transplant could be placed The peritoneal cavity was then opened and the left limb of the pancreas mobilized preserving several of the vessels entering the gland from the splenic pedicle The gland was then transected as closely as possible to its most central part The major pancreatic duct could be seen easily in most cases and was suture ligated The isolated left limb of the pancreas was then mobilized out of the peritoneal cavity taking great care not to tear off the vessels nourishing it from the splenic pedicle The peritoneum was closed about the vessels supplying the transplant exercising care not to damage or constrict these vessels This step in the procedure was extremely critical for too much tension would produce ischemia of the gland The previously prepared rectus compartment was then closed about the transplant Postoperatively the dogs were given tetracycline banthine and nothing but water by mouth for 48 hr Wound edema was usually present for 3 or 4 days but then rapidly subsided Banthine was used because of its suppressive effect on pancreatic secretion[6] After 7 days the dogs were fed standard kennel diets and awaited Stage II

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# ISLET CELL TRANSPLANTATION*

WILLIAM R RUNDLES AND HENRY SWAN

Transplantation of the pancreas or of functioning islet cell tissue has long been envisioned as a way of surgically curing diabetes mellitus To date the problems relative to homografting any tissue have been insurmountable save in special instances such as agammaglobulinemia,[1] monozygotic twins closely inbred animals arterial and corneal transplants and in certain endocrinologically active tissues such as parathyroid

Autografts of various tissues i e thyroid and adrenal[2, 3] have been shown to be successful when implanted in various sites such as muscle subcutaneous space liver spleen brain and serosa of gastrointestinal tract

The object of this investigation was to determine whether functioning islet cells devoid of pancreatic acinar tissue could be transplanted in the same animal A thorough survey of available literature has failed to reveal any reports of transplantation of islet cells *per se*

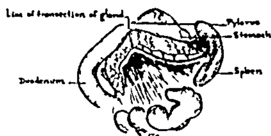
The classic work of Von Mering and Minkowski[4] demonstrated that a transplant of part of the pancreas outside the peritoneal cavity or separated from the duodenum but with its blood supply intact could prevent the development of diabetes after removal of the abdominal pancreas They then removed the transplant and found that glycosuria occurred Later attempts to transplant portions of the pancreas were unsuccessful because of the profound autolytic effect of the pancreatic digestive enzymes Efforts to prepare an active extract from pancreatic tissue with which to treat diabetic patients were also unsuccessful until Banting and Best ligated the pancreatic duct to produce atrophy of the acinar tissue and subsequently prepared an active extract

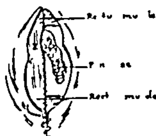
This method of ligating the pancreatic duct to produce acinar atrophy has been adapted for obtaining pancreatic tissue for transplantation The significant feature of this approach is that the pancreatic autograft is essentially devoid of acinar cells yet contains active islets

The following experiments on 18 dogs were performed in the form of staged operations with the objective of first producing a preparation of

*From the Department of Surgery and The Halsted Experimental Laboratory University of Colorado School of Medicine Denver Colo Aided by a grant from The National Institutes of Health United States Public Health Service (GG 3029)

Fig 1

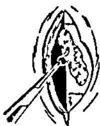


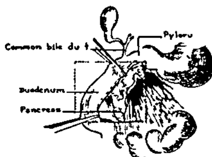



STAGE I

Division of the left limb of the pancreas

Elevation of the left limb to the rectus compartment with blood supply intact






STAGE II

Division of the blood supply to the transplant from below the peritoneum

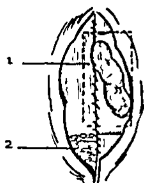
Excision of the remaining pancreas and adjacent segment of the duodenum

islet cells and then observing whether the tissue would survive autotransplantation

## METHOD

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Fig 2




STAGE III

1 Excision of the transplant
2 Transplant is transferred to the right lower quadrant in thin slices



STAGE IV

Excision of the transplanted islet cells to again produce diabetes

**Stage II (see Fig 1)** In approximately 6 to 8 wks Stage II was performed hoping that by this time most of the acinar tissue of the transplant would be atrophic A right paramedian incision was utilized, the abdomen opened and the vascular pedicle going to the left upper quadrant transplant was sectioned A thorough search of the abdominal cavity was made at this time for aberrant pancreatic tissue A complete pancreatectomy was then performed removing all intra abdominal pancreas This necessitated in all cases excision of a segment of duodenum because of the inter relationship of the blood supply of the right limb of the pancreas and the duodenum A conventional type duododuodenostomy was performed just distal to the entrance of the common bile duct

Postoperatively these dogs were given prophylactic penicillin and streptomycin for 4 days and not allowed food until stools were passed Urine was tested with clinitest daily, and a non fasting blood sugar was done during the first post op week to insure absence of hyperglycemia and glycosuria Steatorrhea developed almost immediately and was combatted with Viokase * Entozyme ** tween 80 and a high protein low fat diet with supplemental vitamins and amino acids

**Stage III (see Fig 2)** Within the first 2 wks following Stage II, on dogs that did not become diabetic the next procedure was carried out This consisted of an en bloc excision of the entire left upper quadrant to insure complete removal of the transplant This necessitated complete removal of the entire abdominal wall down to and including peritoneum The transplant was then sectioned into thin (less than 3 mm ) slices and implanted into the lower portion of the right rectus compartment with marking sutures placed for later identification This tissue when examined microscopically showed numerous islet cells and varying degrees of degeneration of the acinar tissue It was quite difficult to secure complete degeneration of all of the acinar elements The defect in the left upper quadrant could be closed in most cases by pulling together the tissue at hand

Immediately following the Stage III operation all dogs immediately became diabetic and the postoperative care then revolved about the treatment

*Viokase (whole raw pancreas) Viobin Corp Monticello Illinois
**Entozyme (pepsin pancreatin bile salts) A H Robins Inc Richmond Va

of the diabetes and the continued nutritional problems caused by the steatorrhea All dogs received regular insulin administered on the basis of urine sugar and blood sugar determinations for the first 24 to 48 hr After this they were switched to NPH insulin given twice daily prior to their twice-daily feedings Close regulation of the diabetes was attempted At intervals insulin dosage was cut sharply to provide a stimulus for autogenic insulin production

Steatorrhea was very troublesome in all dogs which had been pancreatectomized After trying some of the usual pancreatin preparations and fat emulsifiers without satisfactory results a combination was finally found which seemed to be fairly effective in decreasing steatorrhea This consisted of adding 1 to 2 gm of Viokase and 2 Entozyme tablets to each feeding A high protein low fat diet was given and the Viokase powder mixed well with the dog food Somagen*** granules were also added to each feeding plus multiple vitamins and iron Parenteral vitamin B complex and vitamins A and K were also given at intervals

With this routine stools could be reduced to 2 or 3 copious semi fatty ones per day Despite this reduction in steatorrhea a gradual weight loss occurred

**Stage IV (see Fig 2)** The final step in the experiment was the excision of the isotransplant in the right lower quadrant to again produce total diabetes

RESULTS

Eighteen dogs underwent elevation of the left limb of the pancreas into the rectus compartment Three dogs died in the early postoperative period from wound evisceration or peritonitis

Fifteen dogs underwent complete pancreatectomy (Stage II) Of these dogs (Group I) 7 were *not* diabetic indicating that the transplant was producing insulin Eight dogs (Group II) were definitely diabetic after pancreatectomy indicating that the transplant had failed to survive or function The failures were presumed due to interference with the blood supply to the transplant in the first stage procedure These 8 diabetic dogs in Group II served as controls and all demonstrated a well known sequence of events related to acute total pancreatectomy consisting of anorexia vomiting extreme weakness cachexia and lassitude leading to death within 10 days if not given insulin This typical picture in the acutely depancreatized dog is quite characteristic

Of the 7 dogs (Group I) that did *not* become diabetic following pancreatectomy 2 dogs died before the next stage could be done 1 from peritonitis and 1 from distemper

The remaining 5 dogs underwent islet cell transfer from the left upper quadrant to the right lower quadrant (Stage III) One dog failed to become diabetic at this time and was sacrificed feeling that some pancreatic tissue had been left in the peritoneal cavity The other 4 dogs became diabetic immediately after the islet cells were transferred showing that the transplant had been the sole source of insulin production They were then treated with insulin to tide them over until the islet cells could again grow and produce insulin

Insulin could be withdrawn from 2 of these dogs 30 days and 52 days

***Somagen (protein yeast and liver concentrates) Upjohn Co Kalamazoo Michigan

respectively following islet cell transfer The first dog lived without insulin for 5 wks but expired from a fulminating bloody diarrhea before the final stage procedure could be performed The other dog lived without insulin 8 wks and then underwent total excision of the transplant He became rapidly diabetic and expired in diabetic coma on the 16th postoperative day

The remaining 2 dogs have been controlled on decreasing amounts of insulin so that approximately 5 mo following islet cell transfer they require less than 5 units of NPH insulin daily These dogs have not yet undergone the final stage operation

### SUMMARY

1 A series of staged operations was done in an effort to autotransplant functioning islet cell tissue

2 The transplanted tissue was transferred to a new location on the abdominal wall and showed evidence of active function after a latent period of from 30 to 52 days

3 Removal of the autograft after a period of survival without insulin again produced the diabetic state indicating that the graft had been the sole source of insulin

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*Obstetrics and Gynecology*

# THE PROLIFERATIVE CAPACITY *IN VITRO* OF TISSUE EXPLANTED FROM HISTOLOGICALLY BENIGN AND MALIGNANT AREAS OF THE SAME UTERINE CERVIX*

J G MOORE AND W W BRANDKAMP

As previously reported [1] the observation was made that histologically normal epithelium explanted from a cervix containing cancer grew with increased proliferative vigor It is the purpose of the present paper to substantiate the observations made earlier and increase our knowledge of the growth characteristics of the histologically benign areas of the cervix associated with nearby malignancies

## METHOD

Tissue obtained by cervical biopsy was divided into 2 portions 1 for histological section and staining and the other for tissue culture studies The latter portion was explanted aseptically as described in a previous publication [2] and a nutritive medium composed of placental cord serum 40 per cent beef embryo extract 4 per cent and a balanced salt solution (Hanks) 56 per cent added Estimates of growth potential were made from the extent and rapidity of growth Cellular morphology was documented by photomicrographs of the living tissue through the use of the phase microscope and by fixed and stained cultures (hematoxylin and phloxin)

Two areas of each cervix were biopsied One biopsy taken from the suggestive area of the cervical lesion and the other from an expected normal area approximately 1 cm from the site of the lesion The explanted tissue from each area was maintained identically in separate cultures Of patients having no lesion or history of malignancy tissue was explanted in a similar manner—one biopsy from the squamo-columnar junction and the other from a point 1 cm distant on the exocervix Cervical biopsies were taken from 2 types of patients 1) those having known cervical malignancies and 2) those having normal cervices

## RESULTS

In 12 cases of cervical carcinoma in whom comparative cultures of the benign and malignant areas of the same cervix were grown 83 per cent of the cultures of the normal cervical epithelium showed a good/excellent proliferative capacity (Table I) It is perhaps understandable that only 75 per cent of the malignant areas resulted in cultures showing a good/excellent growth response since the malignant areas were often necrotic and inflamed The cells extending from the normal explant (Fig 1) showed a uniformity of structure and pattern of growth whereas those growing from malig

* From the Department of Obstetrics & Gynecology University of California School of Medicine Los Angeles Calif Supported by Grant No C2113 USPHS

*Table 1 Proliferative Capacity of Malignant (A) and Benign (B) Cervical Epithelium from the Same Cervix*

| | PATIENT | DIAGNOSIS | AREA | PROLIFERATIVE CAPACITY |
|---|---|---|---|---|
| 1 | 5 4 | Squamous cell carcinoma | A | No growth |
| | 5 4 | Squamous cell carcinoma | B | Good |
| 2 | 7 4 | Squamous cell carcinoma | A | Excellent |
| | 7 4 | Squamous cell carcinoma | B | Good |
| 3 | 13 4 | Squamous cell carcinoma | A | Excellent |
| | 13 4 | Squamous cell carcinoma | B | Excellent |
| 4 | 14 4 | Squamous cell carcinoma | A | Good |
| | 14 4 | Squamous cell carcinoma | B | Excellent |
| 5 | 16 4 | Squamous cell carcinoma | A | Good |
| | 16 4 | Squamous cell carcinoma | B | Good |
| 6 | 18 4 | Minimal invasion | A | Excellent |
| | 18 4 | Minimal invasion | B | Excellent |
| 7 | 2 6 | Squamous cell carcinoma | A | Excellent |
| | 2 6 | Squamous cell carcinoma | B | Excellent |
| 8 | 4 6 | Squamous cell carcinoma (necrotic | A | No growth |
| | 4 6 | Squamous cell carcinoma tissue) | B | No growth |
| 9 | 7 6 | Squamous cell carcinoma (contaminated | A | No growth |
| | 7 6 | Squamous cell carcinoma 2 months) | B | No growth |
| 10 | 8 6 | Squamous cell carcinoma 1360r X radiation | A | Good |
| | 8 6 | Squamous cell carcinoma | B | Excellent |
| 11 | 9 6 | Squamous cell carcinoma | A | Good |
| | 9 6 | Squamous cell carcinoma | B | Good |
| 12 | 10 6 | Squamous cell carcinoma | A | Excellent |
| | 10 6 | Squamous cell carcinoma | B | Excellent |

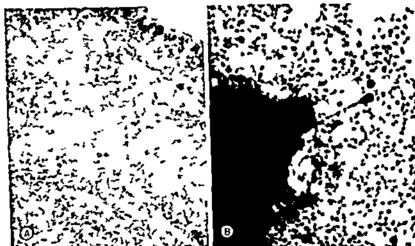
nant explants (Fig 2) displayed marked cellular pleomorphism Comparative photomicrographs of higher magnification (Fig 3) indicate further the varying cell types prevalent in the cultures of malignant tissue as contrasted with the uniformity of the cytoplasmic/nuclear ratio and staining characteristics of the normal autologous tissue In contrast with cultures made from identical biopsies taken from 10 cervices in which no malignancy could be demonstrated only one showed even a minimal growth response (Table 2)

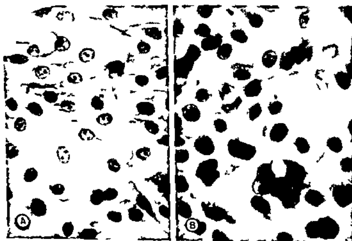
It is noteworthy that work now in progress shows that the malignant areas of questionably malignant cervical lesions (i e carcinoma *in situ et cetera*) grow in culture with equal vigor as frank cervical carcinoma More

*Table 2 Proliferative Capacity of Normal Adult Cervical Epithelium*

| | PATIENT | DIAGNOSIS | PROLIFERATIVE CAPACITY |
|---|---|---|---|
| 1 | 3 3 | Normal Cervix | No Growth |
| 2 | 8 3 | Normal Cervix | No Growth |
| 3 | 11 3 | Normal Cervix | No Growth |
| 4 | 19 3 | Normal Cervix | No Growth |
| 5 | 22 3 | Normal Cervix | No Growth |
| 6 | 27 3 | Normal Cervix | No Growth |
| 7 | 22 5 | Normal Cervix | No Growth |
| 8 | 33 5 | Normal Cervix | No Growth |
| 9 | 41 5 | Normal Cervix | No Growth |
| 10 | 1 6 | Normal Cervix | Very Restricted Growth |


Fig 1 (A) Stained histological section of normal area 80X (B) Stained tissue culture growth 80X


Fig 2 (A) Stained histological section of malignant area from same cervix shown in Fig 1A 80X (B) Stained tissue growth 80X


Fig 3 Comparative photomicrographs of higher magnification of Fig 1B and 2B 200X Note pleomorphism and hyperchromatic cell of malignant area

over in 16 separate experiments so far histologically normal areas of these cervices containing controversial lesions have shown the same vigorous response in 82 per cent of the cultures Also it should be stated that pregnant cervical epithelium shows a definitely increased proliferative vigor Of tissue cultured from 13 such cervices 69 per cent resulted in a definite but uniform growth response None demonstrated such a marked proliferative

capacity as was observed in cultures of malignant or questionably malignant cervical epithelia

### DISCUSSION

The problem of what causes "normal" cervical epithelia when associated with malignancy *to proliferate so vigorously is a most intriguing one* The growth potential of this tissue appears equal to that of malignancy, however its ability to continue at an extended high rate of growth is less marked In these instances the histologic section of the "normal" cervical epithelia revealed no cytologic variation that might justifiably be termed a precancerous condition Additional study concerning the factors contributing to this enhanced growth response of normal tissue associated with malignant tissue appears to be warranted

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## STILL AND MOTION PICTURE HYSTEROSALPINGOGRAPHY*

JOHN A KERNER EARL R MILLER AND ALLAN PALMER

Hysterosalpingography has proved to be one of the most useful diagnostic techniques in gynecology It has been used extensively in our infertility clinic at the University of California and in private practice The technique is useful in demonstrating defects of the corpus and cervix of the uterus *abnormalities of the tubes and movement of the opaque* material in the peritoneal cavity In some cases it appears to be therapeutically beneficial During the last 2 years motion picture studies of the hysterosalpingogram have been instituted making use of an image intensifier and this new work has been most rewarding

### METHOD

To facilitate performing a large number of hysterosalpingograms it was desirable to make our procedures as simple as possible Of several types of apparatus used for injections the most suitable for our purposes was the Riazi Palmer instrument * which has been described by Raoul *Palmer of* France This apparatus is compact and easy to handle (Fig 1) Its features include a metal syringe with screw handle an aneroid manometer which is fastened directly to the syringe and a simple stand A properly made screw

*From the Department of Obstetrics and Gynecology and the Department of Radiology of the University of California School of Medicine San Francisco The image intensifier and camera used for these studies were made available for use through an institutional grant from the American Cancer Society

*Manufactured by Collin et Cie 6 Rue de l'Ecole de Medicin Paris VIe



Fig 1

tip cannula used gently and fit tightly is least painful for the patient Such a cannula makes it possible to dispense with a speculum and provides a means by which one obtains excellent visualization of the endocervix and internal cervical os Cannulas of the Colvin design are made in 3 sizes for our use by the instrument maker at the University of California hospitals

It is customary to use the apparatus for making a series of films as an out patient procedure and to follow the immediate study with a film made 24 hr later

A variety of media has been used and we have come to the conclusion that for our purposes all currently available water soluble media absorb too rapidly Because there is so much information obtained from the 24 hr film the examination should be carried out to at least this length Accordingly a low viscosity form of lipiodol (Ethiodol)* was selected and this agent has been used over 1 000 times in some 600 patients The medium is ideal for 24 hr films yet it is absorbed within 3 to 4 wks The few febrile reactions noted early in the series have been eliminated by the use of antibiotic drugs prophylactically whenever there was suspicion of pelvic inflammatory disease or when more than 150 mm of mercury pressure was necessary No other reactions of importance have been observed

During the last 2 years the fluoroscopic image intensifier has been used as an adjunct to the hysterosalpingogram This apparatus is described in detail elsewhere[2, 3] Briefly by means of an electron-optical system the image intensifier produces a fluoroscopic image several hundred times brighter than the original produced by x ray This makes it feasible to produce satisfactory motion pictures (Fig 2) of a fluoroscopic image and to view the image in subdued light Exposure of both physician and patient to scattered x ray is significantly reduced A most interesting series of motion picture films has

*Supplied for this study by the E Fougera & Co Inc.

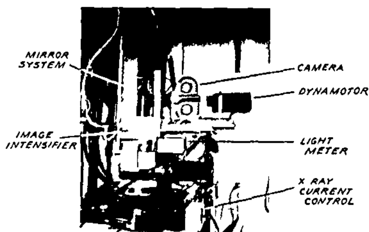



Fig. 2

resulted from the combined use of the image intensifier and the well established technique of Ethiodol injection

## RESULTS

**Findings with the Hysterosalpingogram** The use of hysterosalpingography as a diagnostic procedure has been generally accepted It reveals gross abnormalities of the uterine cavity and tubes In addition the technique often reveals crypts in the endocervix which may represent unexpected foci of infection It may show abnormalities of the internal os not clearly disclosed in any other fashion The 24 hr film gives a clue to abnormalities within the pelvic peritoneal cavity

**Findings with the Motion Picture and Image Intensifier** Although fluoroscopic studies have always been of interest in hysterosalpingography, it has been impossible to re examine any part of the procedure except by occasional "spot" films The motion picture has provided us and other workers[3] with a permanent record of the fluoroscopic study The motion picture has revealed intrauterine pathology which was often missed in the standard film taken when an entire defect or mass was obscured by the medium The motion picture has shown the radiopaque medium during the time it was surrounding a mass or flowing into a defect It also has presented, in a dynamic fashion the physiology of the tube and peritoneal cavity

The Philips Image Intensifier, used so far has a relatively small field and the individual frames of motion picture film do not compare in definition with the "spot" films However it is felt that newer equipment will provide not only a larger field but also clearer pictures

## SUMMARY

A technique of hysterosalpingography and motion pictures in hysterosalpingography has been briefly described The hysterosalpingogram has proven to be invaluable in the study of certain infertile women and as a diagnostic aid in other gynecologic disorders The motion picture made by using the image intensifier becomes a new tool which adds to our understanding of the physiology of the uterus tubes and peritoneal cavity and may become of increasing usefulness in the more routine studies of the pelvic organs

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# CLOSURE OF THE INCOMPETENT CERVIX DURING PREGNANCY*

ROBERT H BARTER JAMES A DUSBABEK H L RIVA AND JOHN PARKS

That the cervix may be inadequate to contain the enlarging products of conception within the uterus has been suggested simultaneously by two gynecologists working in different parts of the world namely Lash in Chicago and Shirodkar in India Lash proposed a plastic operation to correct cervical incompetency and first published his results in 1950[1] although his work dated back to 1911 Shirodkar s purse string procedure did not become known in this country until after his presentation at the International Congress on Gynecology Obstetrics in Geneva Switzerland in 1954[2]

The concept of midpregnancy loss due to cervical incompetence has not been accepted by all obstetricians and gynecologists However there is a small but definite group of patients who have habitual abortion or immature labor after the 16th week of gestation The usual sequence of events is that of painless effacement and dilatation of the cervix with subsequent prolapse and rupture of the membranes The latter is followed by abortion or the delivery of a previable infant

The diagnosis of this condition may be made by radiological means[3] by weekly or biweekly examination of the cervix during a pregnancy in which the cervix manifests incompetency or from the history of repeated late abortions or immature labors always preceded by rupture of the membranes

It is with some reluctance that this new procedure is being reported for the first time in the United States for our results have not been as good as those reported by Shirodkar However with improvement in technique better results are anticipated There has been no desire on the part of the authors to report this procedure without serious forethought[4]

## MATERIAL AND RESULTS

The patients in this series are from the obstetric services at the George Washington University Hospital Walter Reed Army Medical Center D C

* From the Departments of Obstetrics and Gynecology The George Washington University School of Medicine Walter Reed Army Medical Center and D C General Hospital

General Hospital and 1 patient from the Alexandria Hospital Alexandria Virginia * The patients surveyed in this report have been treated during the past 21 months During that time approximately 16 250 patients have been delivered at the first 3 hospitals named It is evident that in any carefully controlled series the number of patients with habitual abortions due to an incompetent cervix will be extremely small The latter point is worthy of repeated emphasis Fascial closure of the cervix to prevent other types of abortion will be uniformly unsuccessful

Sixteen patients are reported at this time These patients have been divided into 2 groups those in whom fascial closure of the cervix was accomplished either with a successful or an unsuccessful outcome and those in whom fascial closure of the cervix has been done during a pregnancy which is still in progress

There were 11 patients in the first group Those 11 patients had had a total of 57 pregnancies including those pregnancies in which closure of the cervix was accomplished Thirty eight of the previous gestations had ended in abortion This group of patients had only 8 living children as a result of their first 46 gestations which represents a salvage rate of 17 4 per cent Of the 11 patients operated upon 5 had surviving infants for a survival rate of 45 4 per cent a significant increase over the previous figure of 17 4 per cent Four of the 5 surviving infants were premature babies but all have done well

In the second group there are 5 patients These 5 patients have had a total of 27 pregnancies 20 of which had resulted in late abortions or the delivery of previable infants Only 2 infants have survived as the result of the first 22 gestations in this group These 5 patients at the present time are in varying stages of late pregnancy and it would appear that all have a fairly good chance of having surviving infants

In both groups there have been a total of 84 gestations Eliminating the 5 patients who are pregnant there has been a total of 79 pregnancies with only 15 surviving infants including the 5 infants who survived after fascial closure of the cervix during pregnancy

If one accepts the figure quoted by Malpas[2] namely that any habitual aborter has a 27 per cent chance of having a successful outcome in any given pregnancy then the figure of 45 4 per cent successful outcome in the first 11 patients would appear to assume importance That percentage figure will be improved when the 5 remaining patients have completed their pregnancies

## TECHNIQUE

The operation is best carried out between the 12th and 18th week of gestation or even later if there has not been too much effacement and dilatation of the cervix The results in this series have been least satisfactory in those patients in whom the cervical dilatation was greater than 4 cm at the time of operation

With the upper vagina adequately exposed by means of retractors, the anterior and posterior lips of the cervix are grasped with ring forceps If there is significant dilatation of the cervix the operation is best done with the patient in rather steep Trendelenberg position An opening is made in the midline of the vaginal mucosa overlying the cervix anteriorly This inci

* Patient of Drs George Speck and Paul Halter Alexandria Virginia

sion is made as high as possible above the lower limits of the cervix and should be 1½″ or 2″ above the most dependent portion of the cervix. The incision is widened laterally so that it is 1½″ long. The bladder is then dissected off the cervix and pushed as high as is possible. The posterior portion of the vaginal mucous membrane is then exposed and a small incision made in the midline 2″ or 2½″ above the external portion of the cervix. A strip of fascia is then threaded to a large aneurysm needle (as designed by Shirodkar). The fascial needle is placed in the anterior incision and it is gently forced posteriorly around the right side of the cervix. The needle should emerge through the incision in the posterior wall of the vagina. The leading end of the fascial strip is pulled through the posterior incision and the aneurysm needle removed. A second aneurysm needle (the mirror image of the first) is then introduced anteriorly and gently forced along the left hand side of the cervix to emerge through the previous posterior incision. The fascial strip is tied to the aneurysm needle and the fascia pulled through to the anterior incision. The fascia is now in position to be drawn around the cervix in the form of a purse string. If the membranes are bulging they will recede into the uterus as the fascia is tightened. The fascial strip is pulled snugly and the two overlapping ends are sutured together with silk, cotton or linen and then anchored to the substance of the cervix. The anterior vaginal incision is closed. The fascia is then anchored to the cervix posteriorly by means of a nonabsorbable suture and the posterior vaginal mucosa is closed with a catgut suture. The patient is kept in bed for 10 to 14 days, after which she is allowed to have a reasonable degree of activity.

Because it was impossible to run a control series in this small group of patients, most of these patients have received adjunctive therapy in the form of vitamin C, progesterone and, in the Walter Reed patients, injections of *Bistrium*.

DISCUSSION

Closure of the incompetent cervix during pregnancy appears to have a very definite place in an extremely small group of patients who have had repeated painless effacement and dilatation of the cervix after the 16th week of gestation. Lash[1] has noted that many of these patients have had some type of trauma to the cervix. These include forcible dilatation of the cervix during a curettement, cervical lacerations from a precipitous labor, a previous traumatic forceps delivery, Durhssen's incisions or a vaginal hysterotomy. The patient should give a typical history of repeated late pregnancy loss which has been initiated by spontaneous rupture of the membranes without antecedent pain. In the patient with this condition the effacement and dilatation of the cervix with protrusion of the membranes may have been observed in a previous pregnancy.

The particular advantage of this operation over the Lash procedure is that it can be accomplished during pregnancy. The principal disadvantage of the Lash operation is that it has to be done in the postabortal state or during a nonpregnant interval. Therefore this procedure has the advantage of being capable of preserving a pregnancy.

The alternative to surgical closure of the cervix in this group of patients is complete bed rest during the last 4 or 5 mo of gestation. Complete bed rest may be accepted by the patient during one gestation, but it will be

accepted rarely in a subsequent gestation Thus a very real advantage of the surgical approach is that the patient may remain ambulatory following the procedure

The fascia used can be homologous (taken from the patient's own thigh) or preserved ox fascia can be used At the present time our preference is for the homologous fascia The use of substances such as polyethylene also has been reported[6]

The patient should be delivered by cesarean section after this procedure to preserve the newly established cervical competency Shirodkar has reported that he has delivered 24 infants from the 19 patients in whom he initially did the procedure In the event that the patient does not desire more children she could be delivered from below However most of these patients with their previous high pregnancy wastage are desirous of having more than one baby thus cesarean section is usually the delivery method of choice

Due to the fact that this method of treatment subjects the patient to not 1 but 2 operative procedures it is again emphasized that it should be undertaken under only the most exacting indications This operation has *no* place in the treatment of repeated early abortions and it is *no* panacea for the treatment of all late abortions


SUMMARY

1 A new procedure for closure of the cervix during pregnancy in the presence of an incompetent cervix has been introduced into this country

2 The procedure was initially developed by Dr V N Shirodkar of Bombay India

3 The operation has been successful in 45 4 per cent of a small series of patients operated upon to date

4 An additional 5 patients who have had the operation are still pregnant and should add to the ultimate success of the operation

5 This operation predisposes the patient to cesarean section

6 This surgery for the correction of habitual late abortions or immature labors is applicable to only a very small highly select group of patients


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# THE EFFECT OF POSITION ON THE COURSE OF THE SECOND STAGE OF LABOR*

MICHAEL NEWTON

Most physicians in this country are accustomed to conducting the second stage of labor with the mother in the lithotomy position Its main advantage is that when used in conjunction with the equipment of the modern delivery room it is easier to ensure asepsis to handle obstetrical hemorrhage to care for the newborn and to terminate the second stage of labor, when necessary, by means of forceps and episiotomy

However looked at from the perspective of history, this position is simply a new fangled fad Sitting kneeling squatting or other positions have been used for countless generations Soranus Gynecology as recently translated by Dr Oswei Temkin contains a section on the construction and use of a midwife s stool or obstetrical chair as was employed in the second century A D In the middle ages also the obstetrical chair was an important adjunct to the practice of midwifery Even today many primitive women continue to deliver their babies in the sitting squatting or other positions We have seen this at the University Hospital in Mississippi in women who have delivered several previous babies in their own cabins Some of them want to kneel on the floor and we have difficulty in persuading them that the delivery table is the proper place

There can be no doubt that modern methods of obstetrical care have increased the safety of both mother and child However in discarding age old positions for the second stage of labor have we adopted a technique which is simply more convenient for the mother s attendants but less mechanically efficient and comfortable for the mother herself?

In considering this question 3 problems present themselves First which position is more efficient for the expulsion of the baby? Secondly if the sitting or squatting position is more efficient can it be adapted to the demands of a modern delivery room? Thirdly does the use of this position improve the management of the second and third stages of labor? The present study represents the preliminary phases of an attempt to answer these questions

During the second stage of labor the mother s voluntary muscles of expulsion are used to augment the uterine contractions in pushing the baby down the lower birth canal The muscles consist of the abdominal muscles the diaphragm and the accessory straining muscles In the sitting position when the back is curved the force of these voluntary muscles is directed towards the pelvis In the supine position with the feet unsupported part of their force is dissipated in pushing the abdominal wall forward An important corollary of this is that when the expulsive muscles contract less efficiently reciprocal relaxation of the muscles of the pelvic floor is also less efficient In addition gravity in the form of the abdominal contents and the weight of the baby is effective in the sitting or squatting position but not in the lithotomy position

The same expulsive muscles are used though to a lesser degree in the everyday functions of defecation and urination These functions are com

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accepted rarely in a subsequent gestation. Thus a very real advantage of the surgical approach is that the patient may remain ambulatory following the procedure.

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## SUMMARY

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6. Bryant, Richard: Personal communication.

There is one more important point to note about the position The head is bent forward by a pillow This helps to put the whole body in a curved line and increases the efficiency of the expulsive effort During contractions the woman should be encouraged to flex her head still further

The modified sitting position is produced by the back rest offers no difficulty in the use of procedures such as cleansing of the perineum control of delivery episiotomy or the application of forceps At first it was thought that anesthesia might represent a problem It does so only if deep general anesthesia is required then the supine position is of great help to the anesthetist However the vast majority of normal obstetrical patients do not require deep anesthesia and when deep anesthesia is necessary the back rest can be put down quickly and easily

Evaluation of the effect of the semi sitting position with the back curved on the second stage of labor represents a difficult problem since many factors enter into it At the present time we have data on the use of the back rest in 86 patients These patients form part of a series of 220 patients followed consecutively at the University Hospital between February and May of 1956 Various physical and psychological data were recorded for each of these patients and a large number of them were interviewed in the immediate postpartum period The details of the experimental method will be published elsewhere Suffice it to say that the data sheets were constructed so that they could be placed on IBM cards for statistical evaluation The sheets were filled in during or immediately after labor by the person who had had most contact with the woman during her labor and delivery i e by the student or intern

Records of the labors of these 86 women seem to us to indicate that the use of the back rest is entirely compatible with convenient and comfortable labor

Most of the women progressed through the second stage of labor with little need for anesthesia Only 14 per cent had caudal spinal or nitrous oxide anesthesia Twenty six per cent found that they needed whiffs of Trilene More than half 60 per cent in fact had no anesthesia at all or merely local infiltration or pudendal block The result of this lack of anesthesia was that only 3 per cent of the women were unconscious or deeply depressed at the time of delivery

The great majority of women were self-controlled and cooperative during delivery Only 7 per cent did not cooperate with requests and directions and only 8 per cent screamed The others responded to directions by the attendants part or all of the time and were either quiet or made moderate vocalizations

The reaction to the baby was mostly a pleasant one Only 8 per cent of the mothers were indifferent None showed active disgust when they first saw the baby The majority smiled a little but 35 per cent were greatly pleased

The obstetrical details of the delivery were similarly normal Forceps were used in less than a quarter of the cases In only 2 per cent were mid forceps necessary

Injury to the perineum was also low Although episiotomies were not performed on 62 per cent of the patients only 6 per cent had second degree tears and there were no third degree tears The third stage of labor was

monly and easily performed in the sitting or squatting position with the back curved. The greater efficiency of these positions will be apparent to anyone who has ever been compelled to make a bowel movement lying flat on a bed pan.

Thus it is reasonable to suppose that childbirth, like urination and defecation, is more efficiently accomplished sitting or squatting with the back curved.

The second problem is that of using these mechanical advantages in such a way that the ease of dealing with obstetrical problems is retained. The use of the traditional obstetrical chair would make it extremely difficult to secure asepsis, to control hemorrhage, or to use obstetrical forceps.

One solution to this problem, as has been done recently, is to raise the obstetrical chair in the air and thus permit the attendant to work under his patient.

A simpler method is to fit a back rest to the conventional delivery table. Such a back rest has been constructed for use in our department. It was made from inexpensive materials by the shop mechanic in our Department of Pharmacology. It is somewhat modified from a similar back rest used at the O'Connor and Franklin Hospitals in San Francisco. The idea for it came from Mrs. Mabel L. Fitzhugh, a physical therapist, who has been greatly interested in education for childbirth in the San Francisco area for the past several years and who is working as a research associate in our department.

Figure 1 shows the Fitzhugh back rest in action. The curve of the mother's back should be noted. The angle of the back rest can be adjusted. Up to the present time we have used an angle of 20 to 40 degrees, but this may not be enough and we plan to increase this angle in future studies.

Until the head begins to show at the introitus, the legs are allowed to be free. In this way the patient can most effectively bear down by pulling on her own legs with her hands just below the knees.

When the stirrups are placed under the legs, they are lowered as far as possible and care is taken to avoid separating the legs too widely. The foot plate is adjusted so that the mother can push hard with her feet but has no pressure under the knees. She can help herself by pulling on the handgrips. Whenever possible we prefer not to restrain the hands.

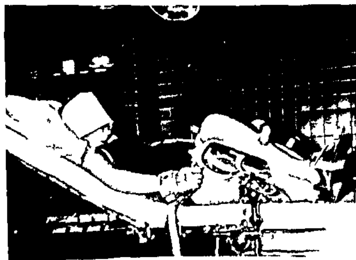


Fig. 1. Position on delivery table in 2nd stage of labor using Fitzhugh back rest.

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rapid with only 5 per cent having retention of the placenta for more than 20 min

These records are quite similar to the other deliveries which took place in the University Hospital at this time There is nothing to suggest that the back rest has an adverse effect on the second or third stages of labor

The data so far available indicate that no disadvantages are apparent from the use of the back rest A number of very favorable clinical impressions have come out of this investigation These are

1 Increased comfort to the woman in the second stage This is particularly noticeable when a patient is raised from the lithotomy to the propped position

2 Increased effectiveness of expulsion This has been noted on a number of occasions when the back rest was raised in women who were making ineffective efforts during the second stage

3 Increased ability of the patient to see and hold her baby immediately after the delivery

4 Increased ability of the woman who has been educated for childbirth to cooperate since she is not lying flat and out of the picture

Because our records show that there appears to be no adverse effects and because of our favorable clinical impression, we plan to investigate the problem further

We plan during the coming year to do a true experimental study on the second stage of labor using comparable test and control groups

SUMMARY

1 The greater efficiency of the propped position with the back curved for the expulsive effort has been discussed with particular reference to the second stage of labor

2 An adjustable back rest has been used for the conduct of the second stage of labor

3 The use of the Fitzhugh back rest is quite compatible with the best methods of modern obstetrical care

4 A preliminary study of the effects of conducting the second stage of labor in the propped position with the back curved has been carried out in 86 women

5 Clinical impressions from this study indicate increased comfort to the mother greater efficiency of the expulsive efforts and considerable advantages in a program of cooperative childbirth

# POSTMATURITY AND FETAL MORTALITY

Edward H Bishop

The subject of postmaturity remains an obstetric enigma in spite of many scientific and clinical investigations When these studies are reviewed they present varying results with controversial and diversified conclusions Two essential points remain unanswered The first is whether or not prolongation of pregnancy actually exists as a pathological state Even when its existence is conceded a second problem still remains that of management The recommended managements vary from a hands off and wait approach of some to suggestions of others for elective termination of pregnancy This latter attitude is typified by the majority of English writers on this subject The pediatric observations of Clifford[1] and the fetal blood oxygen saturation studies of Walker[2] have offered clinical and scientific support to those who advocate interference in order to avoid the supposed hazards of postmaturity Unfortunately these investigations have not answered the problem of the obstetrician He cannot observe the baby before its birth and there are no means by which he may determine prenatally the amount of oxygen being delivered to the infant Instead the clinician must make the diagnosis of postmaturity only by the inexact signs and symptoms which are classically used for determination of the expected date of delivery Probably the most reliable of these is the menstrual history but even this is renowned for its physiologic and pathologic variations The clinician must also as a result of his experience or that of others decide which of the methods of management offers the lesser fetal risk that of waiting for the spontaneous onset of labor or that of interference This clinical study is presented as a step towards solving these problems of the clinician

All patients both ward and private delivered at the Pennsylvania Methodist and Lankenau Hospitals during a period of 1 yr were used as the material for this study These hospitals vary sufficiently in their geographic location type of patient and social group served to offer a cross section and an average sampling of the general population When each patient was registered for delivery at a hospital the expected date of delivery was noted on the hospital records If a patient was delivered more than 14 days after the recorded expected date of delivery she was interviewed personally by the author or by an obstetric resident who had been assigned to this problem at each hospital There were 305 patients who comprised this group of supposedly postmature deliveries However the personal interview revealed that in 118 instances there had been enough previous variation in the menstrual history or enough question regarding the exact time of onset of the last menstrual period to make the diagnosis of postmaturity equivocal These doubtful cases were discarded and this study is concerned with the remaining 187 cases who were considered as definitely postmature from the clinical point of view The incidences of premature delivery mature delivery and postmature delivery are shown in Table 1 The incidence of 3 2 per cent postmaturity is significantly less than that reported in many series using the same definition of delivery 294 days or more after the first day of the last menstrual period This decreased incidence is probably

*Table 1 Incidence of Postmaturity*

| | NO | % |
|---|---|---|
| Premature deliveries | 109 | 7 1 |
| Mature deliveries | 5 164 | 89 7 |
| Postmature deliveries | 187 | 3.2 |

*Table 2 Method of Delivery*

| | ALL CASES | | POSTMATURE | |
|---|---|---|---|---|
| | NO | % | NO | % |
| Spontaneous | 2370 | 41 1 | 61 | 32 6 |
| Low Forceps | 2728 | 47 4 | 94 | 50 3 |
| Mid forceps | 142 | 2 5 | 15 | 8 0 |
| Breech | 178 | 3 1 | 7 | 3 7 |
| Version | 6 | 0 1 | 0 | 0 0 |
| Cesarean | 336 | 5 8 | 10 | 5 4 |
| Total | 5760 | 100 0 | 187 | 100 0 |

*Table 3 Perinatal Mortality*

| | PREMATURE | MATURE | POSTMATURE |
|---|---|---|---|
| Stillbirths | 11 0% | 0 9% | 2 1% |
| Neonatal | 22 3% | 0 3% | 2 1% |
| Total | 33 3% | 1 2% | 4 2% |

a result of having obtained the information directly from the patient rather than from her record alone

A comparison of the methods of delivery of the postmature group with all other deliveries occurring during the same period of time is presented in Table 2 The incidence of delivery by means of major operative obstetrics (cesarean plus mid forceps) was 8 3 per cent in the term group and 13 4 per cent in the postmature group This higher rate is significant but its importance is lessened when one considers that the postmature group consisted of babies of somewhat greater size and weight predicating a longer labor and more difficult delivery The average weight of the post mature baby was 7 7 lbs and 44 per cent of all postmature babies weighed 8 lbs or more This seems adequate reason for the moderate increase in major operative obstetrics

The primary factor which has aroused the concern of many regarding postmaturity is the reported increase in the fetal death rate in this group It is the number of unexplained fetal deaths which prompt many writers to advise termination of pregnancy The perinatal mortality of all categories premature term and postmature in this series is shown in Table 3 The perinatal mortality of 4 2 per cent in the postmature group is significantly higher than that of the mature group and is equally divided between still births and neonatal deaths It is the comparable figures of other authors which have led to the conclusion that pregnancy should be terminated if it continues more than 14 days after the calculated date of delivery However, it is necessary to examine the causes of fetal death before it is possible to reach a similar conclusion from our experience These causes as

determined by both clinical observation and autopsy examination were as follows: 1) Erythroblastosis. 2) Anoxemia associated with difficult delivery. 3) Diaphragmatic hernia and bilateral bronchopneumonia. 4) Intercranial hemorrhage following difficult delivery. 5) Congenital aplasia of the adrenals. 6) Bilateral diaphragmatic hernia. 7) Unknown. Macerated stillborn. 8) Anencephalic monster.

In this series congenital lesions were responsible for the deaths of 4 babies, birth trauma for 2, and erythroblastosis for 1. It is unfair to compare the corrected incidence of 1 death in the postmature group with the uncorrected deaths in the mature group, but it can be stated without equivocation that earlier termination of pregnancy would not and could not have salvaged those babies dying of congenital lesions. It is equally true that only better obstetric judgment rather than termination of pregnancy would have salvaged the 2 that died as a result of birth trauma. In direct contrast one hesitates to estimate what the fetal loss might have been if all of these 187 pregnancies of 294 or more days duration might have been terminated either by induction of labor under unfavorable circumstances or by cesarean section, a procedure which carries with it an increased fetal loss when compared to vaginal delivery. Clifford[1] has reported that among primigravidous patients 26 yr. or older who deliver after 300 days 1 out of every 3 babies die either in utero or after birth. There were 42 patients in this series who fulfilled these qualifications and in this group there were 2 baby deaths rather than the prophesied 14. These 2 deaths were the result of diaphragmatic hernia and intercranial hemorrhage.

## CONCLUSIONS

1. The incidence of postmaturity in this series of 5,760 deliveries is 3.2 per cent.

2. The average birth weight of the postmature baby is slightly above average birth weight of the mature baby.

3. There is an increased incidence of operative obstetrics in the postmature group, probably related to the increased birth weight.

4. Careful examination of the causes of fetal death in the postmature group does not reveal any constant hazard due to prolongation of pregnancy.

5. There does not appear to be any justification for termination of pregnancy because of postmaturity. However, the chances of *increased* baby size must be constantly considered and appropriate obstetric management elected.

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*Table 1 Incidence of Postmaturity*

| | NO | % |
|---|---|---|
| Premature deliveries | 409 | 7 1 |
| Mature deliveries | 5 164 | 89 7 |
| Postmature deliveries | 187 | 3.2 |

*Table 2 Method of Delivery*

| | ALL CASES | | POSTMATURE | |
|---|---|---|---|---|
| | NO | % | NO | % |
| Spontaneous | 2370 | 41 1 | 61 | 32 6 |
| Low Forceps | 2728 | 47 4 | 94 | 50 3 |
| Mid forceps | 142 | 2.5 | 15 | 8 0 |
| Breech | 178 | 3 1 | 7 | 3 7 |
| Version | 6 | 0 1 | 0 | 0 0 |
| Cesarean | 336 | 5 8 | 10 | 5 4 |
| Total | 5760 | 100 0 | 187 | 100 0 |

*Table 3 Perinatal Mortality*

| | PREMATURE | MATURE | POSTMATURE |
|---|---|---|---|
| Stillbirths | 11 0% | 0 9% | 2 1% |
| Neonatal | 22 3% | 0 3% | 2 1% |
| Total | 33 3% | 1 2% | 4 2% |

a result of having obtained the information directly from the patient rather than from her record alone

A comparison of the methods of delivery of the postmature group with all other deliveries occurring during the same period of time is presented in Table 2 The incidence of delivery by means of major operative obstetrics (cesarean plus mid forceps) was 8 3 per cent in the term group and 13 4 per cent in the postmature group This higher rate is significant but its importance is lessened when one considers that the postmature group consisted of babies of somewhat greater size and weight predicating a longer labor and more difficult delivery The average weight of the postmature baby was 7 7 lbs and 44 per cent of all postmature babies weighed 8 lbs or more This seems adequate reason for the moderate increase in major operative obstetrics

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## CONCLUSIONS

1 The incidence of postmaturity in this series of 5 760 deliveries is 3 2 per cent

2 The average birth weight of the postmature baby is slightly above average birth weight of the mature baby

3 There is an increased incidence of operative obstetrics in the postmature group probably related to the increased birth weight

4 Careful examination of the causes of fetal death in the postmature group does not reveal any constant hazard due to prolongation of pregnancy

5 There does not appear to be any justification for termination of pregnancy because of postmaturity However the chances of increased baby size must be constantly considered and appropriate obstetric management elected

## REFERENCES

1 Clifford S H Postmaturity—with placental dysfunction Clinical syndrome and pathologic findings J Pediat S Louis 44 1 13 1954
2 Walker J Foetal anoxemia J Obst Gyn Brit Empire 61 162 180 1954

*Table 1 Incidence of Postmaturity*

| | NO | % |
|---|---|---|
| Premature deliveries | 409 | 7.1 |
| Mature deliveries | 5,164 | 89.7 |
| Postmature deliveries | 187 | 3.2 |

*Table 2 Method of Delivery*

| | ALL CASES | | POSTMATURE | |
|---|---|---|---|---|
| | NO | % | NO | % |
| Spontaneous | 2370 | 41.1 | 61 | 32.6 |
| Low Forceps | 2728 | 47.4 | 94 | 50.3 |
| Mid forceps | 142 | 2.5 | 15 | 8.0 |
| Breech | 178 | 3.1 | 7 | 3.7 |
| Version | 6 | 0.1 | 0 | 0.0 |
| Cesarean | 336 | 5.8 | 10 | 5.4 |
| Total | 5760 | 100.0 | 187 | 100.0 |

*Table 3 Perinatal Mortality*

| | PREMATURE | MATURE | POSTMATURE |
|---|---|---|---|
| Stillbirths | 11.0% | 0.9% | 2.1% |
| Neonatal | 22.3% | 0.3% | 2.1% |
| Total | 33.3% | 1.2% | 4.2% |

a result of having obtained the information directly from the patient rather than from her record alone.

A comparison of the methods of delivery of the postmature group with all other deliveries occurring during the same period of time is presented in Table 2. The incidence of delivery by means of major operative obstetrics (cesarean plus mid forceps) was 8.3 per cent in the term group and 13.4 per cent in the postmature group. This higher rate is significant but its importance is lessened when one considers that the postmature group consisted of babies of somewhat greater size and weight predicating a longer labor and more difficult delivery. The average weight of the postmature baby was 7.7 lbs. and 44 per cent of all postmature babies weighed 8 lbs. or more. This seems adequate reason for the moderate increase in major operative obstetrics.

The primary factor which has aroused the concern of many regarding postmaturity is the reported increase in the fetal death rate in this group. It is the number of unexplained fetal deaths which prompt many writers to advise termination of pregnancy. The perinatal mortality of all categories premature, term and postmature in this series is shown in Table 3. The perinatal mortality of 4.2 per cent in the postmature group is significantly higher than that of the mature group and is equally divided between stillbirths and neonatal deaths. It is the comparable figures of other authors which have led to the conclusion that pregnancy should be terminated if it continues more than 14 days after the calculated date of delivery. However, it is necessary to examine the causes of fetal death before it is possible to reach a similar conclusion from our experience. These causes as

Collections from the football bladder receptacle were made once or twice daily, measured and samples saved. The abdomen was inspected for dryness and the bag carefully inspected for leaks. If a leak was found the output of acid was not computed though the values for pepsin activity were determined.

To verify the functional state of the vagal innervation of the pouch insulin tests were performed on each animal. The volume of gastric juice was measured for two 30 min control periods after which the dog was given 5 or more units of crystalline insulin intravenously. The volume output was again measured at 30 min intervals for 2 hr. During the second or third interval after injection the output nearly doubled if vagus function was intact.

After 30 or more daily observations were recorded the neurosurgical operation was performed. Resections of portions of the cerebral cortex which are presumed to have connections with the hypothalamus and thus may initiate or control vagal impulses were made.

Thirteen intracranial operations were performed on 11 dogs. Bilateral prefrontal lobotomies were carried out on 3 animals and on one of these amputation of the frontal poles was ultimately done. Bilateral cingulate gyrus resection was done twice. Left cingulate gyrus resection was done twice and on one of these dogs the corresponding operation on the right side was later completed. Pituitary resection, left occipital lobe resection and right insula resection were each performed on one dog.

At autopsy the innervation of the pouch was established, the mucosa of the pouch and of the esophagoduodenostomy examined, the brain and the pouch preserved and appropriate microscopic sections were taken for study.

The daily volume of gastric juice was measured, the amount of free and total hydrochloric acid determined by titration with 1/N sodium hydroxide and the peptic activity analyzed by an egg white (digestion turbidometric method). The product of the volume in liters and the free acid in clinical units is the daily output of acid in milliequivalents. In the pepsin analysis the change in turbidity of a standardized egg white solution was measured after a period of digestion at uniform temperature by a sample of gastric juice.

The relationships of egg white concentration, time of digestion and pepsin concentration are described by the equation $dC/dt = kxC$, where C is the relative concentration of protein at time t, k represents the velocity constant proportional to the rate of digestion of egg white and is proportional to the pepsin concentration. The value k is recorded in our records as pepsin activity. It has been computed from the formula $k = 1/(t_2 - t_1) \times \log C_1/C = 0.46 \log C_1/C_2$. $C_1$ and $C_2$, the concentrations of the egg white substrate at the beginning and end of the 5 min digestion period, are read from the standardization curve of the photolometer.

## RESULTS

Table 1 lists the 19 surgical procedures performed on 11 total gastric pouch dogs, the control values for acid and pepsin, the postoperative values after each procedure for prolonged periods, the postoperative values in the first 10 to 14 days after each procedure, the number of measurements

## *Neurosurgery and Neurophysiology*

# THE EFFECT OF CORTICAL AND SUBCORTICAL BRAIN LESIONS UPON GASTRIC SECRETION IN DOGS WITH A VAGUS PRESERVED TOTAL GASTRIC POUCH*

HARRY M. RICHTER, JR., RICHARD A. DAVIS, DANIEL RUGE AND NORMAN T. WALTER

The purpose of this study has been to investigate the possible influence of cerebral cortical and subcortical experimental lesions upon gastric secretion in the dog.

### METHOD

Total gastric pouches were constructed in healthy mongrel dogs of 15 to 25 kg. according to the technique outlined by Dragstedt.[1] Through a mid-line upper abdominal incision the pylorus and proximal duodenum were mobilized, freed of blood vessels for a length of 3 cm., and the duodenum was transected near the pylorus. The pyloric end of the stomach was closed and inverted. The stomach was pulled firmly down from the diaphragm, tensing both vagi and making them readily palpable on and behind the lower esophagus. The circumference of the stomach was dissected 1 cm. below the esophagus deep to the peritoneum and the vagus nerves.

The only technical difficulty with the preparation occurred at the dorsal half of this dissection. If the dissection is conducted at the esophago-gastric junction instead of the lower level just described, one opens the chest and creates a hernia which is difficult to close permanently. The lower esophagus was grasped in a noncrushing clamp and the cardiac end of the stomach transected in the mobilized portion. The cardiac end of the stomach was closed and inverted, the esophageal end, usually bearing 1 cm. of gastric mucosa, was anastomosed to the open bulb of the duodenum. A new stainless steel cannula was inserted into the gastric pouch, sealed with a turn of omentum and brought through a small incision in the abdominal wall to the left of the principal wound. The cannula was secured in its tight and dry track with metal and rubber washers and a ring fitted with a lock screw.

During the first postoperative week the animals were supported with daily subcutaneous injections of 500 cc. or more of 0.9 per cent saline solutions and given prophylactic antibiotics. On the fifth day water was allowed and during the second week milk and ground meat were added to the diet. At the end of this period if alimentation was not adequate gavage feedings were given. If the blood loss was unusually great at operation, 100 to 200 cc. of whole blood was transfused. If food or hair was found in the stomach, they were removed to prevent blockage of the cannula and perforation of the pouch.

*From the Department of Surgery, Northwestern University Medical School. Supported by U.S. Public Health Grant number B-920 (C).

contributing to each average the percentage rise or fall of acid and pepsin after each operation and the statistical significance of each change

In the table the dogs are grouped according to the type of neurosurgical or other operation performed When a dog was subjected to more than one operation a new control series of observations was made before the later operation Hydrochloric acid output is recorded as mEq /day (24 hr) and pepsin concentration as peptic activity from the computations noted above

The study of the first 10 measurements of acid and pepsin during the first 2 postoperative weeks after the neurosurgical procedure was chosen to record the maximal response to stress and to allow for some days lost for record when collection bags were torn

The results listed below as significant are statistically so to the degree indicated in the table The biological meaning of this statistical significance has not been determined

Two dogs were subjected to left cingulate gyrus resection In the acute phase one had an increase in acid output the other showed no change In the chronic phase both showed a significant increase in acid Decreases in pepsin appeared in both acute and chronic periods

Of 3 dogs which had bilateral cingulus resections all showed both early and prolonged decreases in acid output In the acute period pepsin output was slightly decreased in 2 and markedly and significantly increased in the third In the long term effect the pepsin was slightly increased in 2 and markedly increased in the third

Of 2 dogs subjected to bilateral prefrontal lobotomy early acid studies were made in 1 and showed a marked significant decrease The long term effect was a very slight drop in both dogs No significant change was observed in pepsin in the 1 dog studied

One dog which had a left prefrontal lobectomy showed a slight early fall in acid but a long term rise There was no early change in pepsin and chronic pepsin studies were not done in this dog

The dog subjected to bilateral prefrontal lobectomy showed a slight early fall in acid but significant final rise The early decrease in pepsin was not maintained

Two dogs which had left occipital lobectomies showed no significant change in acid values One showed a marked fall and 1 a marked rise in pepsin output

Right insula resection in 1 dog produced a marked early fall in acid but no long term effect Conversely there was no early change in pepsin but after 2 wks a sustained rise

Hypophysectomy caused no change in acid output but produced a prompt and sustained significant fall in pepsin

Four dogs which had replacement of a broken cannula showed an early slight fall in acid output and an early marked fall in pepsin

Vagotomy in 2 dogs following earlier neurosurgical studies showed an immediate marked and sustained fall in both acid and pepsin values

SUMMARY

Neurosurgical procedures were performed in dogs in which total gastric vagus preserved pouches had been prepared

Prefrontal lobotomies prefrontal lobectomies unilateral and bilateral

*Table 1 Gastric Secretory Data**

| DOC | TYPE OF OPERATION | CONTROL HCl | (n) | ACUTE HCl | CHANGE % | P | CHRONIC HCl | (n) | CHANGE % | P | CONTROL PEPSIN | (n) | ACUTE PEPSIN | CHANGE % | P | CHRONIC PEPSIN | (n) | CHANGE % | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 34 | Left singular resection | 58 | (51) | 65.6 | +24 | > 0.05 | 62.7 | (69) | +18 | < 0.05 | 0.054 | (22) | 0.037 | −31 | > 0.05 | 0.046 | (123) | −15 | > 0.05 |
| 56 | Left singular resection | 40.3 | (38) | 41 | +1 | > 0.05 | 50 | (52) | +22 | < 0.05 | | | | | | | | | |
| 16 | Bilateral singular resection | 55.3 | (59) | 52 | −5 | > 0.05 | 31.4 | (41) | −40 | < 0.01 | 0.165 | (19) | 0.141 | −15 | > 0.05 | 0.191 | (87) | +16 | > 0.05 |
| 27 | Bilateral singular resection | 66.1 | (39) | 47.4 | −28 | < 0.01 | 61.7 | (79) | −7 | > 0.05 | 0.159 | (34) | 0.150 | −6 | > 0.05 | 0.175 | (81) | +10 | > 0.05 |
| 34 | Bilateral singular resection | 62.7 | (69) | 35.8 | −46 | < 0.01 | 41.7 | (82) | −33 | < 0.01 | 0.046 | (123) | 0.080 | +74 | < 0.01 | 0.072 | (85) | +56 | < 0.01 |
| 25 | Bilateral prefrontal lobotomy | 50.4 | (49) | 27 | −47 | < 0.01 | 47.6 | (91) | −6 | > 0.05 | 0.156 | (33) | 0.166 | +6 | > 0.05 | 0.171 | (82) | +10 | > 0.05 |
| 26 | Bilateral prefrontal lobotomy | 60.2 | (41) | | | | 55.2 | (31) | −8 | > 0.05 | | | 0.140 | | | 0.142 | (49) | | |
| 25 | Left prefrontal lobectomy | 47.6 | (91) | 42 | −12 | | 69.8 | (98) | +45 | < 0.01 | 0.171 | (82) | 0.173 | +1 | > 0.05 | | | | |
| 24 | Bilateral prefrontal lobectomy | 70.2 | (55) | 63.4 | −9 | > 0.05 | 83.7 | (31) | +27 | < 0.05 | 0.154 | (27) | 0.125 | −19 | < 0.01 | 0.145 | (42) | −6 | > 0.05 |
| 39 | Left occipital lobectomy | 30.4 | (51) | 37 | +22 | (5) | 31.2 | (23) | +13 | > 0.05 | 0.093 | (61) | 0.084 | −10 | (5) | 0.077 | (53) | −17 | < 0.05 |
| 44 | Left occipital lobectomy | 105.4 | (39) | 96.6 | −5 | > 0.05 | 103.6 | (32) | 0 | | 0.144 | (61) | 0.181 | +26 | < 0.05 | 0.182 | (33) | +26 | < 0.01 |
| 47 | Right insula resection | 36 | (46) | 23.6 | −34 | | 33.5 | (63) | −7 | > 0.05 | 0.144 | (71) | 0.151 | +4 | > 0.05 | 0.168 | (53) | +17 | < 0.01 |
| 32 | Pituitary resection | 63.2 | (51) | 63 | 0 | | 66.3 | (80) | +5 | > 0.05 | 0.224 | (66) | 0.112 | −50 | < 0.01 | 0.114 | (101) | −50 | < 0.01 |
| 25 | Replace cannula | 69.8 | (98) | 52.6 | −25 | < 0.01 | | | | | 0.173 | (10) | | | | 0.156 | (126) | −10 | > 0.05 |
| 27 | Replace cannula | 58.5 | (84) | 22 | −62 | (5) | | | | | 0.175 | (81) | 0.089 | −49 | (5) | | | | |
| 32 | Replace cannula | 64.4 | (51) | 59.4 | −9 | > 0.05 | 59.6 | (17) | −9 | > 0.05 | 0.224 | (66) | 0.170 | −24 | < 0.05 | | | | |
| 34 | Replace cannula | 51.7 | (50) | 47.8 | −8 | > 0.05 | | | | | 0.074 | (57) | 0.048 | −35 | < 0.05 | 0.054 | (22) | −27 | < 0.01 |
| 25 | Vagotomy | 69.8 | (98) | 12 | −83 | < 0.01 | 11 | (20) | −84 | < 0.01 | 0.156 | (126) | 0.047 | −70 | < 0.01 | 0.044 | (108) | −72 | < 0.01 |
| 34 | Vagotomy | 41.7 | (82) | 24.9 | −40 | < 0.05 | 29.1 | (80) | −30 | < 0.01 | 0.072 | (85) | 0.029 | −61 | < 0.01 | 0.032 | (41) | −55 | < 0.01 |

*HCl as mEq /24 hr
Pepsin as peptic activity
Parentheses mark number of measurements

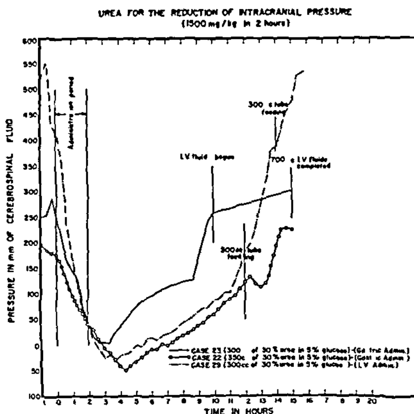



Fig 1 Effect of 1500 mg/kg of urea on cerebrospinal fluid pressure In each case pressure fell to a level which was below normal even though there was a very high initial pressure in 1 of the patients

patients for periods ranging from a few hours to 19 hr In these studies data were also obtained on the following points the effect of hypertonic sucrose and dextrose compared with urea the relation of pressure changes to urinary output the concentration of urea nitrogen in blood urine and cerebrospinal fluid at various intervals following injection

The efficacy of urea for the reduction of intracranial pressure is illustrated in Figure 1 Here are represented the results on 3 patients with intracranial neoplasm each of whom received 1500 mg/kg

The study was conducted preoperatively in 1 patient (Case 23), it was carried out postoperatively in the other 2 The intravenous route was used in Case 29 the gastric route in the other 2 cases All 3 patients were over 70 yr of age A marked effect was seen in each instance The pressure fell to a negative level i e below atmospheric in 2 cases and to the zero level in the third The pressure dropped promptly as illustrated by the fact that the low level of approximately 40 mm was reached *before the 2 hr period of urea administration had been completed* The effect persisted for about 12 hr in each case

The 1500 mg/kg given in the cases just cited represents a large dose and it should be noted that cerebrospinal fluid pressure fell to levels which were below normal even when there was a very high initial pressure Since it has been a constant finding that response to a given dose of urea is greater when initial pressure levels are high it is probably true that a dose

cingulate gyrus resections, left occipital pole resections, and an insular and pituitary resection were done Of these operations only that of bilateral cingulate gyrus resection produced a consistent prolonged reduction in acid secretion In fact it closely approximated the results obtained with vagotomy This suggests that of the areas ablated the cingulate gyrus has *the greater influence on the gastric acid production mediated via the vagus nerves*

In the early period where the stress of surgery played a role the alterations in acid output were generally downward Likewise the initial pepsin determinations were usually decreased It was noted that an increase in pepsin accompanied a very marked acid reduction


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## UREA IN THE MANAGEMENT OF INCREASED INTRACRANIAL PRESSURE*

MANUCHER JAVID PAUL SETTLAGE AND THOMAS MONFORE

Management of intracranial hypertension often presents a difficult problem to the neurosurgeon Many agents have been used for the purpose of pressure reduction none of which has proved to be ideal in all respects The present study concerns the use of hypertonic solutions of urea which we have been investigating for two years at the University of Wisconsin To our knowledge there has been no other study of the effect of urea on intracranial pressure in man A preliminary report[1] describing the early phases of the present study was published in March 1956

To date urea has been administered to 66 patients from the neurosurgical service The greater portion of these were cases of intracranial neoplasm Included also were patients with head injury, hypertensive encephalopathy brain abscess hydrocephalus meningitis cerebrovascular accident and Meniere s disease Doses ranging from 100 mg /kg to 1500 mg /kg were given both by the intravenous and oral routes Intravenous urea has usually been given in 30 per cent solution with 5 per cent dextrose as a solvent Cerebrospinal fluid pressure was measured in approximately half of the

*Division of Neurosurgery Department of Surgery Department of Anatomy University of Wisconsin Medical School Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation Madison Wisconsin

fluid to escape from the lumbar puncture needle. It should be pointed out that the comparison of sucrose and urea was made on an equal volume rather than an equimolar basis. If one had wished to give an amount of 50 per cent sucrose which contained the same number of molecules as 256 cc of 30 per cent urea, it would have been necessary to inject the obviously excessive and dangerous amount of 876 cc.

The evidence which has been collected thus demonstrates conclusively that cerebrospinal fluid pressure may be lowered effectively with the aid of urea. Single doses which are sufficiently large to bring initially elevated pressures down to negative levels are still within the range of safety and are well tolerated. The secondary rise in pressure which has been reported as occurring following administration of 50 per cent dextrose has not been a problem with urea.

Having satisfied ourselves as to the efficacy of urea as an agent for reducing intracranial tension, we turned more recently to the problem of determining suitable schedules for prolonged administration. Our studies have shown that the drop in cerebrospinal fluid pressure is proportional to urine output and urea nitrogen excretion. This indicates that diuresis is the primary mechanism of action in producing the hypotensive effect. Water loss and dehydration cannot be maintained indefinitely, so the problem resolves itself into one of determining what degree of dehydration spread over what period of time will be of greatest net benefit to the patient in terms of reduction of intracranial pressure. We know from experience that repeated doses of urea administered several times daily over a period of weeks continue to be effective. However, since the effect lasts for a matter of 3 to 6 hr. with moderate doses, such a regimen would merely produce intermittent periods of pressure reduction. Is there any way in

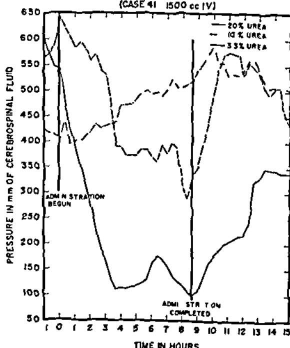



Fig. 3. Comparison of different concentrations of urea solution given over an 8 hr. period by slow intravenous drip (Case 41). The 3.3 per cent solution caused a slight rise in pressure, whereas the 10 per cent and the 20 per cent solutions produced a fall. Excessive dehydration was caused by the 20 per cent solution.



Fig 2 Comparison of the effect of intravenous administration of identical volumes (256 cc) of 50 per cent sucrose and 30 per cent urea (Case 25) In the 2 rows of photographs the condition before injection of the hypertonic agent is illustrated on the left The middle photograph in the upper row represents the maximum reduction following sucrose which occurred 30 min after injection The effect of sucrose had disappeared almost completely within 1 hr as is shown by the third photograph in the upper row The result of injecting urea as seen after an interval of 2 hrs is shown by the lower right photograph The effect was near maximal at this time The crater like area persisted for another hour and the return to the preinjection condition in which the mass was bulging required the lapse of another 8 hrs

of 1500 mg /kg though it is well tolerated is greater than would be needed for clinical purposes Very satisfactory results have been achieved with doses ranging between 500 to 1000 mg /kg

Figure 2 demonstrates the hypotensive effect of urea in another manner and also provides a comparison of the results of the use of 30 per cent urea as opposed to 50 per cent sucrose The three photographs in the upper part of the figure show the patient (Case 25) before and after the administration of 256 cc of 50 per cent sucrose Radical resection of a recurrent right temporal oligodendroglioma had taken place 3 mo prior to the study Pseudomeningocele had developed at the site of subtemporal decompression As may be seen in the center one of the upper 3 photographs (taken at the time when the sucrose effect was maximal) the sugar injection did not produce a significant reduction in the volume of the mass (On another occasion the same volume of 50 per cent dextrose produced an even lesser effect) The 2 photographs in the lower part of the figure illustrate the effect of an identical volume (256 cc to 1000 mg /kg) of 30 per cent urea intravenously The left photo was taken before injection the right one shows how the bulge was converted into a crater like depression by the end of 2 hr The crater persisted for another hour and there was still some noticeable effect of the urea injection at 11 hr On a subsequent occasion it was found necessary in order to reproduce the decompressive effect of the above specified amount of urea to allow 87 cc of cerebrospinal

fluid to escape from the lumbar puncture needle. It should be pointed out that the comparison of sucrose and urea was made on an equal volume rather than an equimolar basis. If one had wished to give an amount of 50 per cent sucrose which contained the same number of molecules as 256 cc of 30 per cent urea, it would have been necessary to inject the obviously excessive and dangerous amount of 876 cc.

The evidence which has been collected thus demonstrates conclusively that cerebrospinal fluid pressure may be lowered effectively with the aid of urea. Single doses which are sufficiently large to bring initially elevated pressures down to negative levels are still within the range of safety and are well tolerated. The secondary rise in pressure which has been reported as occurring following administration of 50 per cent dextrose has not been a problem with urea.

Having satisfied ourselves as to the efficacy of urea as an agent for reducing intracranial tension, we turned more recently to the problem of determining suitable schedules for prolonged administration. Our studies have shown that the drop in cerebrospinal fluid pressure is proportional to urine output and urea nitrogen excretion. This indicates that diuresis is the primary mechanism of action in producing the hypotensive effect. Water loss and dehydration cannot be maintained indefinitely, so the problem resolves itself into one of determining what degree of dehydration spread over what period of time will be of greatest net benefit to the patient in terms of reduction of intracranial pressure. We know from experience that repeated doses of urea administered several times daily over a period of weeks, continue to be effective. However, since the effect lasts for a matter of 3 to 6 hr with moderate doses, such a regimen would merely produce intermittent periods of pressure reduction. Is there any way in

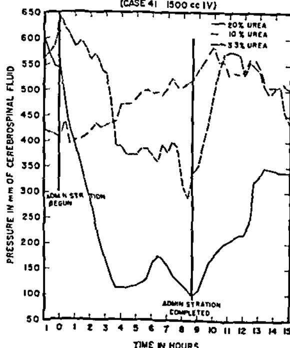



Fig 3 Comparison of different concentrations of urea solution given over an 8 hr period by slow intravenous drip (Case 41). The 3.3 per cent solution caused a slight rise in pressure whereas the 10 per cent and the 20 per cent solutions produced a fall. Excessive dehydration was caused by the 20 per cent solution.

which more sustained effect could be obtained without serious difficulties due to dehydration?

Figure 3 illustrates one attack on this problem On 3 successive occasions the same patient (Case 11) received 1500 cc of urea solution by intravenous drip during an 8 hr period Different concentrations of urea were used on the different occasions, namely, 3 3 per cent, 10 0 per cent and 20 0 per cent Surgical removal of left fronto temporal glioblastoma had been carried out 6 mo prior to the time of these studies and the patient had been comatose for about 1 mo The curves indicate that the 10 per cent and 20 per cent solutions produced marked pressure reduction whereas the 3 3 per cent solution actually appeared to cause a slight increase in pressure The 15 hr urinary output following the 3 3 per cent 10 0 per cent and 20 0 per cent solutions were 1700 cc 3000 cc and 6700 cc respectively It should be emphasized that the 1500 cc dose of 20 per cent urea (1400 mg /kg) which was administered over an 8 hr period is definitely not a recommended dose It produced a very marked reduction in pressure and maintained it well (approximately 50 per cent of the initial pressure) but it caused a marked state of dehydration which it was necessary to combat with vigorous measures at the conclusion of the study

The data in Figure 3 suggest that an optimal concentration of urea for prolonged administration may be somewhere between 3 3 per cent and 10 per cent An ideal concentration probably would be one which though administered in large volumes would nevertheless produce a small net fluid loss Such a concentration might serve to maintain a state of decreased pressure with minimal adverse effects from dehydration The search for such a concentration is continuing

Therapeutic application of urea was not the primary purpose in the early phases of this investigation, since it was important first to test the efficacy and safety of the drug However, after it had been demonstrated that urea could be administered in large amounts without untoward effect we began employing it routinely in cases of acute postoperative cerebral edema and in those preoperative cases when temporary control of intra cranial pressure was essential Dramatic improvement when urea was life saving occurred on several such occasions

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# ELECTRICAL STIMULATION OF THE HUMAN CEREBELLUM*

NICHOLAS WETZEL AND R. S. SNIDER

Stimulation of the cerebellum in the human has not been reported frequently. We have been able to stimulate the intact human cerebellum in 9 cases. Our results differ from those obtained by Pool in the only previous report, but the circumstances of stimulation and types of stimulation are not comparable.

The present work arose from earlier basic studies on the cerebellum. As is well known, there are certain so-called sensory areas, such as tactile, auditory, and visual, which exist in the cerebellum and which are related to similar functional areas of the cerebrum. Electrical stimulation of these areas in animals may cause alternations in the EEG in certain cerebral areas. Changes produced by such stimulation may cause a reduction of frequency, i.e., slowing, or, under a differing set of experimental conditions or stimuli, low voltage fast activity may be initiated. Electrical stimulation of the cerebellum has also been shown to stop seizure activity initiated by electrical stimulation of the cortex. There is an adequate anatomical foundation for cerebellar projections to the motor and sensory areas of the cerebrum as well as to the reticular areas of the brain stem. Because of this background, we have felt that an experimental approach to these problems in the human was justified. Accordingly we have, whenever practicable, stimulated the exposed cerebellum and have recorded changes in the electroencephalogram through conventional scalp electrodes. The patients studied have had various portions of the cerebellum exposed in routine neurosurgical procedures and have been free from signs and symptoms of cerebellar disease. Some of the procedures have been done entirely with a local anesthetic of 1 per cent xylocaine, but most have been operated upon with the local anesthetic plus nitrous oxide-oxygen or trilene through an endotracheal tube for the convenience and comfort of all concerned. Anesthesia has been kept at such a level that there are minimal EEG alterations.

A Grass Model III C electroencephalograph was used with scalp electrodes placed on the available scalp. Stimuli were applied to the pia overlying the available cerebellar cortex using a bipolar electrode with electrical stimuli being generated by a Grass model 3A stimulator at parameters varying between 5 and 25 volts and frequencies between 1 and 300/sec. Points which caused changes in the EEG were suitably marked and photographs then taken. Observation of any motor movements were also made, as were changes in blood pressure, pulse, and respiratory rates.

In one instance we were able to expose most of the anterior lobe. In most of the others the exposure was such that the tonsillar portion of the paramedian lobule and the regions immediately adjacent to them and/or the flocculonodular area were available.

The EEG effects which were observed usually lasted for several seconds following withdrawal of the stimuli and were free of any accompanying motor movement. Single shock stimuli were ineffective and it was necessary to faradize the cerebellar cortex for from 1 to 5 sec. It was found that elec-

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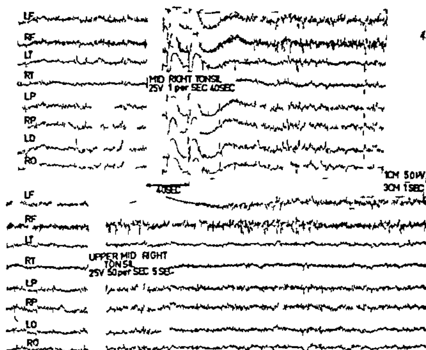



Fig 1 Electrical stimulation of pial surface of right tonsil of cerebellum Voltage duration and frequency are given Scalp recording from following sites LF & RF left and right frontal LT & RT left and right temporal LP & RP left and right parietal *LO & RO left and right occipital Note enhancement of LF & RF* voltage as a result of tonsilar stimulation

trical stimulation of the tonsillar area could enhance the amplitude of the fast activity recorded from the cerebral hemispheres In 2 cases this enhancement was limited to the frontal regions of the cerebral cortex Although enhancement of fast activity could be observed following anterior lobe

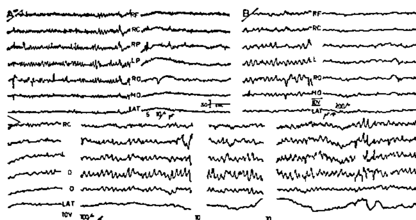



Fig 2 Electrical stimulation of pial surface of anterior lobe In (A) note that there is a reduction of slow waves (B) same as (A) While in (C) there is increased slowing Note removal of 10 sec strips from continuous tracing which can have fast activity superimposed on it Scalp leads over cerebral hemispheres were as follows RF RC RP and RO right frontal central parietal and occipital areas LP LAT and MO left parietal anterior temporal and motor (precentral) areas

stimulation the usual situation was the changing of fast activity to higher voltage slow waves especially in the range of 1 to 5 sec

In no case were we able to induce so called seizure activity in the cerebral cortex as a result of cerebellar stimulation Figures 1 and 2 are examples of the 2 kinds of EEG alterations which were observed

Although some variation in the results occurred in the various cases it is our impression that the following statements are justified

1 Electrical stimulation of the anterior lobe induced reduction of voltage and slowing of the cerebral EEG patterns

2 Stimulation of the tonsillar portion of the paramedian lobule produced increased voltage and frequency of EEG patterns

3 Single shock stimuli were ineffective but faradization often produced effects which lasted many seconds in the poststimulatory period

4 It was not possible to produce cerebral seizure activity by cerebellar stimulation

5 The above effects were elicited without any visible signs of motor response

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## CEREBRAL METABOLIC STUDIES OF HYPOTHERMIA IN THE HUMAN*

John E Adams Henry Elliot Violette C Sutherland Edwin J Wylie and Robert D Dunbar

Although hypothermia is being used with increasing frequency in cardiac vascular and neurological surgery many of the basic physiological changes occurring in the hypothermic state are as yet incompletely understood The nature of and tolerance for the profound acidosis resulting from hypothermia for example and the alteration in cerebral metabolic processes in relationship to the utilization of oxygen and glucose have not yet been adequately determined That cerebral tissues have a lowered oxygen requirement in the hypothermic state is now well established on the basis of both *in vitro* and *in vivo* studies Whether this decreased demand for oxygen is related merely to a lower cerebral metabolic rate or whether there is an actual alteration in the oxidative processes is unknown The majority of previous investigations concerning cerebral metabolic changes in hypothermia have been done in animals and scant human data are available The present study was undertaken because of the foregoing considerations

The patients studied were either undergoing neurosurgical procedures or were being operated upon for aortic aneurysms They were anesthetized with ether which was the anesthetic agent used throughout the surgical

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procedure unless the degree of hypothermia made additional anesthesia unnecessary Preliminary premedication consisted of demerol and atropine When the anesthetic level anticipated as necessary for the surgical procedure was attained a control cerebral blood flow study was done It soon became apparent that it was necessary to administer the nitrous oxide mixture for 15 min to obtain equilibration between the brain and the cerebral venous blood in the hypothermic state The control blood flow study was therefore also extended to 15 min instead of terminating it after the usual 10 min The patients were then cooled in a refrigeration blanket the arterial needle being kept in place to provide continuous measurement of mean arterial blood pressure When the desired temperature was attained a second cerebral blood flow determination was carried out A closed gas exchange system was utilized so that an equivalent amount of ether was administered both during the control and the experimental blood flow study *It was found that a mixture of 15 per cent nitrous oxide and 85 per* cent oxygen yielded better uptake curves than did the mixture usually used for cerebral blood flow determinations

Blood oxygen and carbon dioxide analyses were made in duplicate by the manometric technique and Van Slyke and Neill [1] as modified by Kety and Schmidt [2] These analyses were made at ordinary room temperature It is probable that for greater accuracy the analyses should be made at temperatures corresponding to the temperature of the blood at the time the sample is obtained We also assumed possibly incorrectly that the partition coefficient remains at unity at low temperature as Kety[3] has shown it to be at normal temperatures Glucose was determined by the iodimetric titration method of Somogyi [4] Glutamic acid and glutamine were measured by the microbiologic method of Harper [5] and lactic acid by a modification of the method by Miller and Muntz [6]

The values for cerebral blood flow and the cerebral metabolic rates for oxygen carbon dioxide etc are expressed in cc /100 gm of brain/min The values for glucose lactic acid glutamic acid and glutamine are expressed in mg /100 gm of brain/min

The results for cerebral blood flow and metabolic rates are tabulated in Table 1 A positive sign represents uptake by the brain a negative sign represents release by the brain Hypothermia caused a reduction in cerebral blood flow in all but 2 patients (H K & H C) In these 2 the mean arterial blood pressure was higher at the lowered temperature whereas a significant drop occurred in all others Consequently it seems probable that the systemic blood pressure is the most important single factor controlling blood flow at hypothermic temperatures A significant increase in cerebral vascular resistance associated with hypothermia occurred Whether this was due to decrease in caliber of cerebral vessels or to increased viscosity of blood cannot be stated The cerebral metabolic rate for oxygen was lowered The high $CMRO_2$ shown for patient M R in the control period may be attributable to the patient s age since it is probable that prepubertal children have higher cerebral metabolic rates than adults It is apparent that utilization of glucose decreases at hypothermic temperatures although the values given are not statistically significant The expected release of lactic acid by the brain in the control period occurred in all but 2 patients This release was less at hypothermic levels

*Table 1*

| | CONTROL | | | | | | | | | | EXPERIMENTAL | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PATIENT | CBF | CMRO | CMRCO | CVR | RQ | CMR GLUCOSE | CMR LACTIC | CMR GLUTAC | CMR GLUTAM | TC | CBF | $CMRO_2$ | CMRCO | CVR | RQ | CMR GLUCOSE | CMR LACTIC | CMR GLUTAC | CMR GLUTAM |
| M. R. 5 | 6.8 | +1.9 | −1.7 | 1.12 | 0.95 | | | | | 29.0 | 26.2 | +1.4 | −0.73 | 3.20 | 0.52 | | | | |
| A. C. 44 | [illegible]1.6 | +3.2 | −3.0 | 1.2 | 0.93 | +1.15 | −0.9 | 1.2 | +0.9 | 29.0 | 27.2 | +1.07 | −0.48 | 3.52 | 0.49 | −3.16 | −2.54 | 0 | +0.16 |
| R. M. 16 | 18.5 | +2.3 | −1.9 | 1.2 | 0.88 | +1.6 | −3.4 | | | 30.0 | 30.8 | +1.2 | −0.73 | 2.10 | 0.60 | −2.52 | −0.9 | | |
| I. B. 72 | 19.6 | +2.8 | −2.4 | 1.66 | 0.87 | +3.9 | −2.8 | +0.4 | +1.1 | 29.5 | 13.0 | +2.2 | −1.82 | 1.93 | 0.84 | −2.62 | −0.7 | 0 | −0.10 |
| J. I. 67 | 55.9 | +2.5 | −3.5 | 1.2 | 1.4 | +6.7 | +1.9 | +0.21 | +0.18 | 29.0 | 30.0 | +0.4 | −0.19 | 2.53 | 1.2 | −0.58 | +0.24 | +0.03 | −0.24 |
| H. K. 55 | 47.51 | +1.3 | −1.7 | 1.96 | 1.32 | +6.84 | −3.6 | −0.37 | +0.73 | 29.5 | 51.5 | +1.2 | −1.95 | 2.11 | 1.5 | +5.31 | −1.34 | +0.03 | −0.36 |
| R. C. 58 | 60.2 | +1.5 | −2.2 | 1.19 | 1.46 | +1.6 | −0.4 | +0.12 | +0.61 | 29.5 | 39.4 | +0.7 | −0.49 | 2.07 | 0.64 | +7.24 | +2.53 | 0 | −0.51 |
| H. C. 67 | 41.6 | +2.3 | −2.3 | 1.63 | 1.0 | +1.78 | +1.8 | | | 31 | 58.0 | +2.1 | −1.84 | 1.03 | 1.1 | +4.58 | +0.23 | | |
| MEAN | 51.5 | +2.60 | −2.71 | 1.43 | 1.10 | +1.93 | −1.05 | +0.31 | +0.71 | | 38.3 | 1.27 | −1.09 | 2.35 | 0.85 | +1.13 | −0.35 | +0.01 | −0.29 |
| Standard dev. of MEAN | ±6.09 | ±1.01 | ±0.91 | ±0.23 | ±0.7 | ±1.6 | ±2.08 | ±0.16 | ±0.13 | | ±3.1 | ±0.68 | ±0.76 | ±0.75 | ±0.55 | ±2.5 | ±1.6 | ±0.02 | ±0.14 |

*Table 2 Arterio Venous Differences*

| | $O_2$ | $CO_2$ | GLUCOSE | LACTIC ACID | GLUTAMIC ACID | GLUTAMINE | $O_2$ | $CO_2$ | GLUCOSE | LACTIC ACID | GLUTAMIC ACID | GLUTAMINE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | +4.40 | −3.99 | +7.33 | −2.1 | −0.11 | −0.61 | +3.37 | −2.75 | +2.63 | −0.98 | −0.03 | −0.71 |
| S | ±0.3 | ±0.2 | ±1.3 | ±1.1 | ±0.11 | ±0.32 | ±0.1 | ±0.08 | ±0.9 | ±0.7 | ±0.008 | ±0.21 |

*Table 3 Absolute Blood Levels*

| | CONTROL | | EXPERIMENTAL | | 10 NORMALS | |
|---|---|---|---|---|---|---|
| | A | V | A | V | A | V |
| $O_2$ Vol % | 18.65 | 11.71 | 20.91 | 16.87 | 16.5 | 11.3 |
| $CO_2$ Vol % | 48.39 | 52.36 | 44.96 | 47.22 | 48.5 | 53.9 |
| Glucose Mg % | 114.8 | 138.7 | 192.9 | 187.2 | 102.3 | 93.9 |
| Lactic Acid Mg % | 11.3 | 13.1 | 19.3 | 20.2 | 6.1 | 7.4 |
| Glut. Acid Mg % | 0.70 | 0.66 | 0.26 | 0.29 | 0.15 | 1.5 |
| Glutamine Mg % | 8.59 | 8.12 | 6.12 | 6.81 | 9.2 | 9.6 |

In several patients cerebral blood flow could not be calculated due to inadequate uptake curves for nitrous oxide. Arteriovenous differences are therefore shown for the total group of 12 patients in Table 2. In general the A-V differences correspond with the cerebral metabolic rates in Table 1 and statistically are more convincing.

The mean values for the absolute levels of the various moieties studied are listed in Table 3. It is uncertain whether the higher values for oxygen indicate increased solubility of gases at lower temperature or whether they represent a metabolic alteration. Insofar as glucose is concerned, it has been shown that plasma glucose levels are increased by hypothermia. The striking increase in blood levels for lactic acid under hypothermia is of great interest. We do not believe that this increase was caused by shivering since in most instances shivering was well controlled by the anesthetic agent.

DISCUSSION

These preliminary results in humans corroborate the findings of others that at hypothermic levels there is a decrease in cerebral blood flow and in the cerebral utilization of oxygen and glucose. Alterations in the arterio-venous oxygen levels in general were related to the rate of cerebral blood flow but a decrease did occur with falling temperatures. Our findings are therefore in agreement with those of Bering *et al.*[7] and at variance with the results obtained by Rosomoff[8] who reported that the arteriovenous oxygen levels remain constant at reduced temperatures. In instances where the mean systemic arterial blood pressure either did not fall or rose at the level of lowered body temperature maintained in the experimental study, the cerebral blood flow did not drop and similarly there was little change in the cerebral metabolic rates for oxygen.

The fact that hypothermia produces high blood levels for lactic acid is of interest and the source of this lactic acid as yet has not been determined. We have carried out a few determinations of blood pH and have found, as others have, that the pH drops to remarkably low levels (7.0 to 7.2). It has

been suggested that this metabolic acidosis is caused by an increased $pCO_2$. Although our data are incomplete they suggest that the acidosis may be related in part to the high blood level of lactic acid since at the measured level of pH lactic acid, not lactate is present.

The fact that there is a definite decrease in the liberation of lactic acid from the brain in the hypothermic state and in some instances a utilization of lactic acid raises the possibility that anaerobic oxidative processes may be operative. Our findings in regard to lactic acid levels are at variance with those of Lougheed and Kahn[9] who observed no change in lactic acid levels as long as their animals were not anoxic. We feel certain that our patients were not anoxic not only on the basis of their appearance but also because of the blood oxygen and $CO_2$ levels.

Further studies are in progress to elucidate some of the problems raised by these findings.


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## INTRACRANIAL IMPLANTATION OF LIQUID PLASTIC IN THE EXPERIMENTAL ANIMAL (MACAQUE) POSSIBLE CLINICAL APPLICATION IN SURGICAL MANAGEMENT OF INTRACRANIAL ANEURYSMS*

CURWOOD R HUNTER FRANK H MAYFIELD BERT H McBRIDE AND HENRY E LATTINVILLE

The purpose of this work is the search for a relatively innocuous liquid or semi liquid agent which might be used intracranially in reinforcing saccular aneurysms without occlusion of the parent vessel and with minimal cerebral scarring effect.

* From The Christ Hospital Research Institute Cincinnati Ohio

### METHOD

Pure methyl methacrylate or polyethylene, the plastic chosen for this study, was dissolved in 1 of several organic solvents and implanted in 11 experimental animals (Macaque). These agents included propyl acetate, ethyl acetate, trichlorethylene, acetone, acetone with dicetyl phosphate and chloroform.

Trephination in the temporal area was carried out in each animal. In some the liquefied plastic was placed in the subdural space about the optic nerve and olfactory tract or in the Sylvian fissure. In others a cerebral defect was created in the inferior frontal and anterior superior temporal lobes measuring 1.5 cm., sparing the middle cerebral complex which was deliberately left to bridge this space. These vessels were then embedded by filling the cerebral defect with liquid plastic.

### RESULTS

**Methyl Methacrylate and Acetone.** 1) No. 1108. This combination was instilled about the right optic nerve, along the frontal base and in the Sylvian fissure. On sacrifice 5½ wks. later India ink irrigation of the common carotid artery on the right side was carried out. Examination of the brain disclosed visualization of the entire cerebral vascular tree without evidence of thrombosis of major vessels. The plastic presented a yellowish white appearance and was flint-like in consistency. It was loosely attached by filmy adhesions to an area of softening extending 0.5 cm. in all directions.

2) No. 1505. Methyl methacrylate and acetone was instilled into a cerebral defect on the right side measuring 1.5 cm. in diameter about the middle cerebral vessels which were deliberately left to bridge this space. On sacrifice 5 wks. later India ink irrigation of the common carotid artery on the right side was carried out. Examination of the brain disclosed no evidence of thrombosis of the major vessels traversing the cuff of plastic placed about them in the Sylvian region. The plastic was densely involved in scar tissue, presented a craterred appearance and was flint-like in consistency.

These two animals survived without evidence of neurological disability. The combination of methyl methacrylate and acetone, while difficult to handle in the liquid state, congealed quickly, the surface first, the interior of the mass remaining liquefied. This characteristic permitted molding of the compound into a spherical shape which could then be cut with scissors and placed as a cuff about the middle cerebral vessels.

**Methyl Methacrylate and Acetone with Dicetyl Phosphate.** 1) No. 1158. This combination was instilled about the right optic nerve and olfactory tract and in the Sylvian fissure in the subdural space. On sacrifice 8 mo. later India ink irrigation of the common carotid artery on the right side was carried out. Examination of the brain disclosed visualization of the entire cerebral vascular tree without evidence of thrombosis of major vessels. The plastic presented a yellowish white appearance and was flint-like in consistency. Filmy adhesions bound it to the pia-arachnoid.

2) No. 1216. Methyl methacrylate and acetone with dicetyl phosphate was instilled into a cerebral defect on the right side measuring 2 cm. in diameter, the middle cerebral vessels bridging this space and consequently completely embedded in the plastic. This animal was sacrificed 6 wks. later

following which India ink irrigation of the common carotid artery on the right side was carried out Examination of the brain disclosed visualization of the entire cerebral vascular tree without evidence of thrombosis of major vessels embedded in the plastic The plastic yellowish white in appearance and flint like in consistency was densely involved in scar the fibrosis response considerably greater in degree than that seen with methyl methacrylate and acetone These 2 animals presented no neurological abnormalities during the survival period

The physical characteristics of methyl methacrylate and acetone with diethyl phosphate vary in only 1 major respect from methyl methacrylate with acetone The former appears to be more liquid and from a technical standpoint is more easily handled However it hardens as quickly as methyl methacrylate and acetone

**Methyl Methacrylate and Tri Chlorethylene** 1) No 4806 This combination was instilled about the right optic nerve the olfactory tract and in the Sylvian area in the subdural space On sacrifice 6 wks later India ink irrigation of the common carotid artery on the right side was carried out Subsequent examination of the brain disclosed no evidence of thrombosis of major vessels However extensive softening of the frontal and temporal lobes was apparent The plastic conforming to its environment had precipitated a dense adhesive process and presented a yellowish white cratered appearance

2) No 1703 Methyl methacrylate and tri chlorethylene was instilled into a 1 5 cm defect involving the inferior frontal and anterior temporal lobes on the right side the middle cerebral vessels left to bridge the space and consequently completely incorporated in the plastic On sacrifice 6 wks later India ink irrigation of the common carotid artery on the right side was carried out Examination of the brain disclosed visualization of the entire cerebral vascular tree without evidence of thrombosis of major vessels The plastic was densely incorporated in scar tissue the fibrosis response considerably greater than with either of the 2 preceding plastic solvent combinations Additionally softening of the adjacent brain substance extended approximately 1 cm in all directions

These 2 animals presented a stormy postoperative course generating large quantities of fluid beneath the scalp at the site of operation In each instance right pupillary dilatation and moderate paresis of the left extremities was present though improving at the end of the survival periods The combination of methyl methacrylate and tri chlorethylene is easily handled technically since it remains liquified for an extended period of time In each of the above instances the material had not solidified as of approximately 30 min after instillation

**Methyl Methacrylate and Propyl Acetate** 1) No 4679 This combination was instilled about the right optic nerve olfactory tract and in the Sylvian area subdurally On sacrifice 6 wks later India ink irrigation of the common carotid artery on the right side was carried out Examination of the brain disclosed visualization of the cerebral vascular tree without evidence of thrombosis of major vessels The plastic which presented a yellowish appearance was densely bound to the adjacent structures by scar tissue Additionally softening in the inferior frontal and anterior temporal area was apparent

2) No 4756 Methyl methacrylate and propyl acetate was instilled into a 1 5 cm defect involving the inferior frontal and anterior temporal region encompassing the middle cerebral vessels left to bridge this space On sacrifice 6 wks later India ink irrigation of the common carotid artery on the right side was carried out Examination of the cerebral vascular tree showed no evidence of thrombosis of major vessels The plastic densely involved in scar tissue and surrounded by an extensive area of softening presented a yellowish appearance was cratered and irregular, and flint like in consistency

These 2 animals were quite ill for several days and weakness of moderate degree of the left extremities was noted Considerable quantities of fluid generated beneath the scalp at the operative site At the end of their survival periods almost full recovery from paresis of the left extremities had occurred

The combination of methyl methacrylate and propyl acetate because of a prolonged liquid phase is easily manageable from a technical standpoint However as of 15 min after instillation in either of the above instances no solidification had occurred and the wounds in each instance were closed without waiting for congealing

**Methyl Methacrylate and Ethyl Acetate** No 4640 This combination was instilled about the optic nerve olfactory tract and in the Sylvian area subdurally on the right side On sacrifice 6 wks later India ink irrigation of the common carotid artery on the right side was carried out Examination of the brain disclosed visualization of the entire cerebral vascular tree without evidence of thrombosis of major vessels The plastic presenting a yellowish white appearance and flint like consistency was densely bound to the adjacent structures by adhesions and softening of moderate degree involved the inferior frontal and anterior temporal lobes

This animal s postoperative course was also somewhat stormy Right pupillary dilatation and paresis of the left extremities of mild to moderate degree was evident However at the end of its survival period it had completely recovered The combination of methyl methacrylate and ethyl acetate congeals slowly and as of approximately 15 min after instillation had not hardened

**Methyl Methacrylate, Acetone and Tincture Merthiolate** No 4507 This combination was instilled into a 1 5 cm cerebral defect involving the inferior frontal and anterior temporal area on the right side about the middle cerebral vessels left bridging this space This animal partially recovered from anesthesia but then regressed and died 24 hr later Postmortem examination after India ink irrigation of the cerebral vascular tree through the right common carotid artery disclosed no evidence of thrombosis of major vessels However approximately 10 cc of subdural fluid of xanthrochromic character was encountered It was felt that this accounted for the animal s demise The plastic had completely solidified presented a pink appearance and its surface was smooth unlike the cratered irregular appearance of specimens left in the animal for several weeks

***Methyl Methacrylate and Chloroform*** *No 4512* This combination was instilled in the left inguinal area about the femoral artery vein and nerve Additionally a small amount was instilled into the Sylvian area subdurally on the left side Methyl methacrylate and acetone was instilled subdurally

in the Sylvian area on the right side and also about the right femoral artery, vein and nerve in the inguinal region During the first 4 days following surgery the animal remained lethargic and inactive though gradually improving On the fifth day the inguinal wounds interrupted bilaterally, and on the eighth day, the animal generally improving but with considerable drainage from its inguinal wounds was returned to the operating room The left femoral artery and vein were found grossly thrombosed and marked tissue reaction surrounded the plastic block On the right side (acetone) the femoral artery and vein were intact Only minimal tissue reaction had occurred although the wound was obviously infected One week later the animal died apparently from intracranial complications and was discarded without our knowledge

## SUMMARY AND CONCLUSIONS

Methyl methacrylate dissolved in 1 of several organic solvents was implanted intracranially in a series of 11 monkeys Death occurred in 2 (chloroform and acetone with tincture of mehthiolate) No neurological sequelae were observed in the 2 animals in which methyl methacrylate and acetone, or in the 2 animals in which methyl methacrylate and acetone with dicetyl phosphate had been implanted The remaining 5 exhibited either transient neurological deficits or sequelae which were reduced to a minimal character by the time of sacrifice

Least local reaction occurred in the case of the acetone combination A slightly greater response resulted with acetone and dicetyl phosphate Marked sclerosis occurred with the remainder of the solvents used The presumption is made that intracranial implantation of chloroform compound would produce a severe reaction based on observations of femoral artery and vein occlusion and subjacent sclerosis of extreme degree Though no interruption of the pia arachnoid barrier occurred in subdural implantation softening of variable degree involved the adjacent cerebral tissue Least reaction occurred with acetone A much greater degree of softening occurred with the other solvents In no instance in the 9 surviving animals was there evidence of thrombotic occlusion of major vessels lying either adjacent to or traversing masses of plastic Patency of the cerebral vascular tree was best demonstrated by postmortem India ink injections

From a technical standpoint the acetone combination was most difficult to handle because of stickiness and jelly like consistency The remaining solvents produced a more easily manageable mass because of a more liquid character However they congeal slowly because of reduced volatility of the organic solvent It is believed that this reduced volatility prolonged the irritating effect of the organic solvent accounting for the greater degree of reaction observed on cerebral tissue especially when the pia arachnoid barrier is interrupted With the acetone combinations high volatility of the solvent permits rapid hardening of the plastic and consequently a much shorter duration of contact of the solvent with the cerebral tissue

It is recognized that experience with these solvents is limited However these preliminary results suggest acetone or perhaps acetone with dicetyl phosphate for increased scarring effect as the least irritating of the organic solvents Furthermore since thrombosis of cerebral vessels lying adjacent to or traversing masses of plastic does not appear to occur it is considered

surgically feasible to encompass intracranial saccular aneurysms as a reinforcing measure where sacrifice of the parent vessel might be complicated by severe neurological disability or in those instances where ligation of the aneurysm is followed in a high percentage of cases by thrombotic occlusion of the parent vessel

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## SUPRAPINEAL RECESS*

ORLANDO J ANDY AND JAMES S BROWNE

Deformities in the configuration of the posterior limits of the third ventricle, secondary to both infra and supratentorial tumors, has been given a great deal of attention However very little study has been given to its configuration in patients without tumors thus lending to the occasional difficulty in recognizing the pathologic patterns

The posterior wall of the third ventricle beginning inferiorly, consists of the aditus of the aqueduct of Sylvius, posterior commissure, pineal recess habenular commissure and the suprapineal recess The suprapineal recess consists of an eventration or diverticulum of the ventricular epithelium and usually contributes to a major portion of the posterior wall A membranous pouch should lend itself very well to displacements and deformities secondary to nearby lesions and thus be of diagnostic value However, under normal conditions membranous diverticula present a variety of patterns and thus in this location may deter from its diagnostic value In this study, emphasis is placed on characteristics of the suprapineal recess under conditions not complicated by tumors

Factors being evaluated are (1) length and (2) height of the recess (3) angle between the recess and the aqueduct and (4) distance between the recess and aqueduct

### METHOD

This study is based on 31 pneumoencephalograms Patients varied in sex race and age (3 to 61 Fig 2) They were selected from the neurological and neurosurgical services of a Veterans and a University Hospital Under local anesthesia pneumoencephalograms were done in the sitting position with the neck anteflexed approximately 45 degrees A lateral x ray was taken at a tube plate distance of 10′ after injecting 10 cc of air

Outline drawings were made of the aqueduct and the posterior wall of the 3rd ventricle (Fig 1) and from these 4 measurements (Fig 2) relative to the suprapineal recess were determined as follows

**(D — — D )** Height of suprapineal recess a measurement between the upper and lower lips of its mouth This mouth is arbitrarily determined by a line running through the habenular commissure perpendicular to Reid s base line

*Division of Neurosurgery University of Mississippi Medical Center and Veterans Administration Hospital Jackson Mississippi

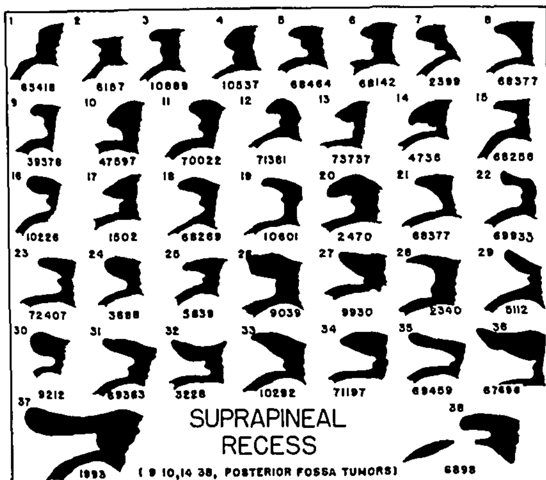



Fig 1 Tracings of the posterior wall of the 3rd ventricle and rostral aqueduct The first 37 cases are in order of increased length of suprapineal recess Cases 14 and 38 represent pontine gliomas Cases 9 and 10 represent a mid line cerebellar astrocytoma and a 4th ventricular cyst respectively

(C — — C) Length of the suprapineal recess the distance from a point mid way between (D — — D) to the most distal portion of the diverticulum

(A) Suprapineal recess aqueduct angle is determined by tangents to the most ventral surface of the recess and the most dorsal surface of the aqueduct with the pineal recess as its point of origin

(B — — B) Suprapineal recess aqueduct distance measurement between the 2 points of tangency as determined in (A)

RESULTS

Considerable variation in the size shape and position of the suprapineal recess was found in patients without space occupying intracranial lesions These variations appeared to be independent of the age sex and race of the patient

The shape varied from a blunt or very shallow eventration (Fig 1 1) to an elongated sac (Fig 1 37) The elongated recesses either curve upward around the splenium of the corpus callosum downward or remain straight Its configuration varied from a narrow to a very wide sac (Fig 1) Length

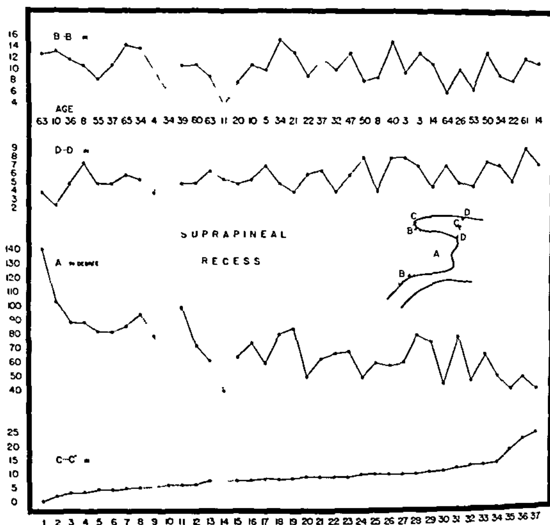



Fig 2 Three measurements in mm and 1 in degree as illustrated in the insert are plotted from the air study of 37 patients Cases 9 10 and 14 have posterior fossa lesions the rest are normal They are arranged in sequence of increased suprapineal recess length (C — — C′) length of recess (A) angle between recess and aqueduct (D — — D′) height of recess (B — — B′) recess aqueduct distance

(Fig 2 C — — C) of the recess ranged from 1 to 24 mm Height (Fig 2 D — — D) varied from 2 5 to 9 mm The recess aqueduct distance (Fig 2 B — — B′) varied from 6 to 16 mm The angle (Fig 2 A) as previously described between the recess and the aqueduct varied from 40 to 140° As the length of the recess increases there is a concomitant decrease in the angle (Fig 2) There is also a suggestive concomitant decrease in the recess aqueduct distance (B — — B) however it is not as marked as the angle alteration The height of the pineal recess is the most stable measurement which undergoes very little change with changes in the pouch s length However there may be a tendency to increased height with increased length In order to demonstrate deviations from the 34 normals 4 pathological cases have been added (9 10 14 and 38 Figs 1 and 2) Cases 14 and 38 are pontine gliomas which considered in their respective positions in the chart (Fig 2) according to recess length show marked decrease in angle size and recess aqueduct distance in comparison to the normal range Case 38 according to recess length should be in the same position as 31 and

would show an angle of 20 degrees thus being completely out of line with the apparent normal trend of the angle curve. Case 10 is a cyst of the 4th ventricle with obvious deviations from the general trend of the curves as shown in Fig 2. Case 9 is a child with an astrocytoma of the 4th ventricle which is presented as an exception since it did not produce detectable changes by the criteria presented above. However 1 yr later the child returned and was found to have very extensive changes in this region (Courtesy of Dr Charles Neill)

## DISCUSSION

Others[1,2] have attempted to use the suprapineal recess as a measurement reference point in the diagnosis and localization of both infra and supra tentorial lesions. Wilson and Lutz[2] have drawn a line along the base of the anterior fossa to the anterior clinoid and another from the latter to the suprapineal recess. This angle was normally 140 degrees. Epstein[1] made measurements from the suprapineal recess to the anterior inferior angle of the 3rd ventricle. This was found to be 4.2 to 4.7 cm. Measurements were also made from the recess to the aditus which ranged from 0.5 to 0.6 cm. Furthermore it is stated that no changes were found in the latter measurements in cases of posterior fossa tumors. He cites Ecker's[3] article as lending support to his thesis.

To the present authors it seems that measurements as suggested by Epstein and Wilson and Lutz presuppose that the suprapineal recess and structures immediately surrounding it in the brain stem act as a unit and move as a mass when displaced. The anatomical differences such as consistency, shape and contents of the structures under question make this supposition improbable. Detailed measurements of the suprapineal recess with reference to its immediately surrounding structures reveals that these structures do not move en mass. Application of the 4 criteria of measurement to the pathological ventricular patterns published by others[3,4,5,6] support these observations.

## SUMMARY

The pneumoencephalographic size, shape and position of the suprapineal recess was described and illustrated with reference to its immediately surrounding structures in 34 patients interpreted as having normal air studies. The suprapineal recess in this group presented a great deal of variation. However 4 measurements of this recess are presented in the form of a graph which can be employed to detect deviations from the expected normal.

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# INHIBITION OF ACTIVITY OF CORTICAL MOTOR NEURONES*

CHOH-LUH LI

It is known that the motor cortex receives fiber projections from the nucleus ventralis lateralis of the thalamus. The latter in turn derives its afferent supply from the red nucleus and more directly from the cerebellum. It is also believed that the globus pallidus also connects with the nucleus ventralis lateralis. Considering the multiple connections of the red nucleus with the tectum, zona incerta, subthalamic nucleus and substantia nigra, and of the globus pallidus with the striatum, one would assume that the nucleus ventralis lateralis would serve as an important relay station to the motor cortex and would exert considerable influence upon the activity of the cortical motor neurones. For instance, lesions in the cerebellum give rise to hypotonia, asthenia and asynergic manifestations. Similarly, diseases of the basal nuclei are frequently accompanied by undesirable movements.

On the basis of clinical observations and the anatomical connections of these subcortical structures, it was postulated that certain neuronal pathways may be significant in relation to motor function.[1]

This report consists of the results from a series of experiments in which the effect of the discharge from the nucleus ventralis lateralis upon the activity of the cortical motor neurones was investigated.

*In all experiments electrical stimulation was applied to the nucleus ventralis lateralis* and to the motor cortex of the cat. The recording electrode which was capable of detecting action potentials from single cortical cells was a glass pipette having an outside diameter of about 0.5 micron at the tip and filled with 3 M KCl solution. The microelectrode, which was inserted into the motor cortex, was then connected to the amplifier-oscilloscope recording unit. Recording of the slower electrical activity adjacent to the cortical cells under study was simultaneously carried out by means of a large electrode on the cortical surface.

When a microelectrode was inserted into the grey cortex it recorded spike action potentials of the single cortical neurones. The spikes recorded were variable in shape, presumably due to the location of the tip of the recording electrode in reference to the surface membrane of the cell.

Thus, if the tip was outside but very near to the cell membrane, spikes which were predominantly negative in electrical sign were recorded. If the tip was in direct contact with the cell membrane, it recorded positive-negative spikes. And if the tip was situated inside a cell, a monophasic positive spike was recorded.[2]

When a cortical unit was discharging "spontaneously" in prolonged trains, a single stimulus to nucleus ventralis lateralis would arrest the discharge of the unit for as long as 100 msec., after which the discharge of the unit recurred. The temporary arrest of "spontaneous" unit spike activity in response to synaptic volleys seemed to be a common occurrence since it was observed not only in the motor cortex but also in the visual and somatosensory cortex.

*However, in the motor cortex when spike activity occurred only in response to stimulation*, it was found that the spike response was also suppressed by a preceding stimulus to the nucleus ventralis lateralis. This is in

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contrast to the observations obtained in the somatosensory cortex where the neuronal responses were facilitated by preceding stimulation of an unspecific thalamic nucleus.[3]

Further the slow potential response evoked by direct stimulation of the motor cortex was also suppressed by a preceding stimulus applied to this thalamic nucleus. Thus when stimulation of the motor cortex elicited a slow potential response and a unit spike discharge, a preceding thalamic volley would cause a reduction in amplitude of the slow potential and disappearance of the unit spike. The inhibitory effect would persist for 40–80 msec.

In conclusion, it has been shown that the discharge of nucleus ventralis lateralis of the thalamus inhibits the activity of the cortical neurones in the motor cortex. Since the principal efferent pathway for voluntary movement has its origin in the motor cortex, it seems logical to suppose that the function of this thalamic nucleus is primarily inhibitory or regulatory in relation to motor activity.

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# CERTAIN EFFECTS OF PERMANENT PITUITARY STALK INTERRUPTION IN THE HUMAN WITH MAMMARY CANCER*

GEORGE EHNI AND NYLENE E. ECKLES

It has been abundantly shown in several species that when the pars distalis is deprived of functional contact with the hypothalamus, it becomes dormant or at best operates at such a depressed level as to be incapable of sustaining certain of its endocrine dependencies. Ablation of pituitary function achieved by hypophysectomy has been shown to exert a favorable effect upon certain patients who have advanced metastatic mammary cancer. Since isolation of the human pituitary gland by permanent interruption of its stalk appeared likely to quench its function with less technical difficulty than attends gland removal and possibly with more certainty of functional ablation, it was decided to subject a small series of humans suffering from advanced metastatic mammary cancer to pituitary stalk interruption. This

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With the technical assistance of Jacqueline Claus in the hormone assays and Fern Garrett in the tests of thyroid function.

were accomplished by means of a right subfrontal approach followed by section of the pituitary stalk and placement of a roughly circular polyethylene stamp about 1 4 cm in diameter upon the diaphragma sella centered over the distal end of the cut stalk

Of the 6 patients herein reported 5 had been previously treated with castration and/or exogenous steroids and had either failed to show response or were in relapse following a period of improvement In the remaining case pituitary stalk section was the initial treatment for the metastatic disease This patient was the youngest of the group She had an extremely short history (1 mo post mastectomy) and displayed widespread osteolytic metastasis with anemia This latter patient was the only one menstruating at the time of stalk section

Cortisone administration during and after surgery was given according to need rather than pre arranged plan Four patients received no cortisone before or during the operation but had it started in doses of 25 mg every 6 hr upon completion of the surgical procedure Of these 4 3 experienced serious hypotension during the first 12 hr following the operation and all responded to intravenously administered hydrocortisone Two patients received 150 mg of cortisone intramuscularly prior to the operation and 50 mg every 4 to 6 hr thereafter One of these patients developed significant hypotension within the first 12 hr following stalk section and required intravenous hydrocortisone

All patients have required maintenance doses of 25 mg cortisone daily for optimum well being although for purposes of test it has been possible to withdraw cortisone from each while under observation in the hospital for periods of from 1 to 4 wks Four of the 6 patients have required pitressin for the control of some degree of presumably permanent diabetes insipidus No other endocrine products or dietary measures for control of electrolytes have been used to date though it is expected that additional replacement therapy may become necessary as end-organ failure (such as the thyroid) progresses

The course of the neoplastic disease was gauged by serial roentgenograms 24 hr urinary calcium excretions serum calcium levels serum alkaline phosphatase studies, hemoglobin determinations and by photographs and measurements of tumor masses The effects upon thyroidal adrenal and ovarian functions were also assayed

## RESULTS

**Effects Upon the Neoplasm** Table 1 displays the essential clinical data on the 6 patients comprising this report It is to be noted that the longest period of observation is 7 mo so that no conclusion can be drawn as to whether benefits deriving from this operation compare favorably in duration with those produced by hypophysectomy adrenalectomy or cortisone therapy The clinical result in the first patient appears at this time however to compare favorably with the best responses produced in other instances of metastatic breast cancer by these other procedures This patient is completely devoid of subjective complaint of any kind has gained 21 lbs in weight has developed no new skull or other lesions and the previously developed osteolytic areas throughout her skeleton have shown a marked blastic response Prior to the operation her 24 hr urinary calcium excretions

*Table 1 Clinical Data on Six Patients with Metastatic Mammary Carcinoma Submitted to Pituitary Stalk Section*

| PATIENT AGE | METASTATIC SITES | CLINICAL STATUS | ENDOCRINE STATUS | SURVIVAL | AFTER STALK SECTION CAPABILITIES | TUMOR CHANGES |
|---|---|---|---|---|---|---|
| 1 31 | Skeletal (lytic) | Bedridden bone pain vomiting anemia | Intact Menstruating | 7 months | Working pain free | No new lesions Strong blastic response in skeletal lesions Eunemia 24 hr urinary calcium fall from 510 mg to 37 mg at 24th week |
| 2 52 | Skeletal (lytic) | Barely ambulatory bone pain | 2 years after menopause | 5 months | Unchanged | Skeletal lesions advance Hypercalcinuria persists |
| 3 37 | Pleural skeletal (lytic) | Barely ambulatory bone pain | Surgical castrate | 4 months | Working pain free | No new lesions Beginning blastic response in old ones 24 hr urinary calcium fell from 165 mg to 25 50 95 mg |
| 4 46 | Pleural nodal chest wall | Ambulatory painful swollen arm | Surgical castrate | 3½ months | Bedridden near death | Progression in all lesions |
| 5 46 | Skeletal subdermal | Bone pain vomiting ambulatory | Surgical castrate | 3 months | Working pain free | No new lesions Early blastic response in old ones Subcutaneous masses smaller 24 hr urinary calcium fall from 208 mg to 44 mg |
| 6 58 | Peritoneal hepatic nodal skeletal (blastic) | Barely ambulatory vomiting ascites | Surgical castrate | Dead 2¼ mo after operation | | All lesions advanced |

averaged 510 mg but since stalk section the value has *consistently* ranged between 30 and 50 mg The third and fifth patients show calcium excretion reductions of the same order They have developed no new skeletal or other lesions during the short period of postoperative *observation* and the blastic response noted roentgenographically in the already existing skeletal lesions though present is of a lower order in magnitude *than* in the first case Three of the 6 patients 1 of whom had skeletal *metastases* seemingly comparable in every way to those in patient Number 1 derived no benefit from the operation by any measurable criterion

**Effects Upon Target Organ Physiology** Thyroid *function* has been evaluated by determination of the 24 hr uptake of orally administered $I^{131}$ and by

determination of protein bound iodine For only 2 of our cases do we believe that the period of observation has been long enough to have significance Patient Number 1 in whom the best clinical result to date was obtained and who has been longest under study shows no appreciable change in either the $I^{131}$ uptake or the PBI Contrasting with this patient is our Number 2 who was not clinically benefited by the operation but who showed at the end of 4 mo an $I^{131}$ uptake of only 4 per cent as contrasted with an initial level of 33 per cent The diminution in her protein bound iodine was similarly striking Observations in the remaining 4 patients suggest that thyroid function is decreasing in all but at a slower rate than reported by others for hypophysectomy

The change in absolute eosinophil count was measured in response to ACTH (25 units given as intravenous infusion over 8 hr) before and after stalk section The drops were normal before surgery but 1 mo after it there was little or no change in the number of circulating eosinophiles during the 8 hr test A moderate eosinopenia occurred when ACTH stimulation was repeated on the second day

The 4 hr glucose tolerance test done at monthly intervals after stalk section reveals extreme flattening of the pretreatment curve This increased sensitivity to endogenous insulin is paralleled by a response to intravenously administered insulin (6 units) following which subconvulsive sugar levels resulted Change in glucose tolerance as measured by these tests parallels that recorded by others for the hypophysectomized state

Plasma corticosteroid levels and the 24 hr urinary excretion of corticosteroids were determined pre and postoperatively after withdrawal for 48 hr or more of the exogenous cortisone and determinations were repeated on successive days following administration of 25 units ACTH by 8 hr intravenous infusion The result in all cases was the same that is, there occurred a drop from normal values of both urinary and plasma corticosteroids to almost unmeasurably small levels following stalk section This occurred within 3 wks after surgery During the 3 day ACTH stimulation test the increase in corticosteroid values of plasma and in 24 hr urine collections on the first day was negligible on the second day showed a slight increase in a few instances only and on the third day in all cases approached normal values

Patient Number 1 had normally functioning ovaries at the time of stalk section Endometrial biopsy was done 3 and 6 mo following operation The material on both occasions showed glandular and stromal hyperplasia of the proliferative type Vaginal bleeding has not appeared This evidence of follicular stimulation without luteinization is not explainable by our material at the present time

## CONCLUSIONS

In a small series of patients with disseminated mammary cancer subjected to permanent pituitary stalk interruption the procedure provided amelioration of the disease of the same order of frequency and magnitude as hypophysectomy During the 2 to 7 months of postoperative observation thyroidal *depression in some degree has been noted in 5 of the 6 cases* All patients showed marked postoperative depression of adrenal function comparable to that reported for patients following hypophysectomy or pituitary necrosis ACTH administration over several days evoked adrenocortical ac

tivity in all subjects tested. Cortisone withdrawal while the patient was hospitalized for periods of 1 to 4 wks. was tolerated but comfort and feeling of well being were adversely affected. In the 1 patient having normally functioning ovaries before stalk section, cyclic bleeding has not reappeared but the endometrium shows estrogenic stimulation. This patient demonstrates the most dramatic regression of neoplasm, and the concept of estrogen dependency determining breast cancer response is in her case at least unsupported.

These preliminary observations suggest that permanent pituitary stalk interruption merits more intensive study both as palliative treatment for metastatic breast cancer, and as a means of advancing understanding of the physiology of neoplasia.

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## AN EVALUATION OF HYPAQUE SODIUM (WIN 8308-3) FOR CEREBRAL ANGIOGRAPHY*

D. W. LINDNER, F. A. MARTIN, J. E. WEBSTER AND E. S. GURDJIAN

Since the acceptance of cerebral angiography as a relatively safe diagnostic procedure, the merits of various media and the techniques of their administration have been reported. The purpose of this study was to evaluate Hypaque Sodium (WIN 8308-3) as a contrast medium, comparing it with other media, i.e., Diodrast, Thorotrast, Urokon and Renographin.

### METHOD

Twenty-two dogs were used in the study. Under nembutal anesthesia, polyethylene catheters were placed in the femoral vein and carotid artery. Injections of the various contrast media were made via the carotid catheter, varying the amount, concentration and rate of injection. Then following the method of Broman and Olsson[1] for analysis of disturbances of the blood brain barrier, 100 cc. of a 0.5 per cent solution of trypan blue was given via the femoral vein over a 10 to 15 min. interval. After the injection of the dye the cerebral vessels were flushed with 200 to 300 cc. of saline and the animal immediately perfused with 10 per cent formalin for fixation. Following perfusion, the cranium was removed and the dura opened. The head with the exposed brain *in situ* was immersed in fresh 10 per cent formalin for 24 hr., at which time the brain was removed intact, sectioned and examined.

Observations were made upon the blood pressure, pulse, respiration and pupil size during and immediately following the injections.

The radiographic densities of similar concentrations and amounts of the various media were compared.

After evaluation of the laboratory data, percutaneous carotid angiography was performed on 260 patients using Hypaque as the contrast medium.

* From the Wayne State University Neurosurgical Service, Grace Hospital and Detroit Memorial Hospital, Detroit, Michigan. Aided by the Kresge Foundation.

determination of protein bound iodine. For only 2 of our cases do we believe that the period of observation has been long enough to have significance. Patient Number 1, in whom the best clinical result to date was obtained and who has been longest under study, shows no appreciable change in either the $I^{131}$ uptake or the PBI. Contrasting with this patient is our Number 2 who was not clinically benefited by the operation but who showed at the end of 4 mo. an $I^{131}$ uptake of only 4 per cent as contrasted with an initial level of 33 per cent. The diminution in her protein bound iodine was similarly striking. Observations in the remaining 4 patients suggest that thyroid function is decreasing in all but at a slower rate than reported by others for hypophysectomy.

The change in absolute eosinophil count was measured in response to ACTH (25 units given as intravenous infusion over 8 hr.) before and after stalk section. The drops were normal before surgery but 1 mo. after it there was little or no change in the number of circulating eosinophiles during the 8 hr. test. A moderate eosinopenia occurred when ACTH stimulation was repeated on the second day.

The 4 hr. glucose tolerance test done at monthly intervals after stalk section reveals extreme flattening of the pretreatment curve. This increased sensitivity to endogenous insulin is paralleled by a response to intravenously administered insulin (6 units) following which subconvulsive sugar levels resulted. Change in glucose tolerance as measured by these tests parallels that recorded by others for the hypophysectomized state.

Plasma corticosteroid levels and the 24 hr. urinary excretion of corticosteroids were determined pre- and postoperatively after withdrawal for 48 hr. or more of the exogenous cortisone and determinations were repeated on successive days following administration of 25 units ACTH by 8 hr. intravenous infusion. The result in all cases was the same, that is, there occurred a drop from normal values of both urinary and plasma corticosteroids to almost unmeasurably small levels following stalk section. This occurred within 3 wks. after surgery. During the 3 day ACTH stimulation test, the increase in *corticosteroid values of plasma and in 24 hr. urine collections on the* first day was negligible, on the second day showed a slight increase in a few instances only, and on the third day in all cases approached normal values.

Patient Number 1 had normally functioning ovaries at the time of stalk section. Endometrial biopsy was done 3 and 6 mo. following operation. The material on both occasions showed glandular and stromal hyperplasia of the proliferative type. Vaginal bleeding has not appeared. This evidence of follicular stimulation without luteinization is not explainable by our material at the present time.

## CONCLUSIONS

In a small series of patients with disseminated mammary cancer subjected to permanent pituitary stalk interruption, the procedure provided amelioration of the disease of the same order of frequency and magnitude as hypophysectomy. During the 2 to 7 months of postoperative observation thyroidal depression in some degree has been noted in 5 of the 6 cases. All patients showed marked postoperative depression of adrenal function comparable to that reported for patients following hypophysectomy or pituitary necrosis. ACTH administration over several days evoked adrenocortical re-

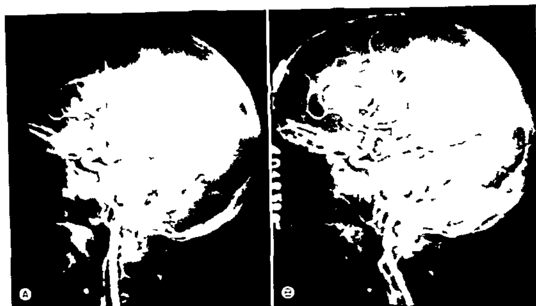


Fig 1

patients were evaluated when the procedure was done under local anesthesia The patients were questioned regarding pain and discomfort during and immediately following the injections A majority complained of some paresthesias or dysesthesias over the face and scalp (mainly a burning pain) usually on the side of the injection This was accentuated when a thrombosis of the internal carotid artery was present and the medium was shunted into the external carotid artery

Two patients expired within 12 hr following angiography One a 16 yr old white male was admitted shortly after the onset of a sudden hemiplegia An angiogram using 2 injections of 6 cc each of 25 per cent Hypaque was normal The patient expired suddenly 11 hr later At autopsy a large acute parietal lobe hematoma was found A second patient a 41 yr old white female died 6 hr after a vertebral angiogram using Hypaque The autopsy



Fig 2

Initially 6 cc of a 25 per cent solution was used per injection Eventually a 50 per cent solution was used in all cases in quantities of 10 to 25 cc per injection (Usually 4 injections of 10 to 12 cc each were used for bilateral angiography) Either local or sodium pentothal anesthesia was employed in the procedures

RESULTS

**Animal Experiments** The immediate effects of rapid injection of Hypaque into the carotid artery of the dog were 1) transient apnea followed by temporary increase in respiration 2) temporary slowing of the pulse 3) temporary dilatation of the pupil on the side of the injection and 4) occasional movements of the facial musculature These effects were also observed when other media were employed and varied in intensity with the different media and their concentrations

*Animals were given Hypaque in amounts varying up to 4 injections* of 20 cc each In none was there gross staining by trypan blue in the cut sections of the brains In the brains of the animals receiving Diodrast and Urokon gross staining was present often limited to the side of the injection The amount of staining varied with the amount and concentration of contrast material used Minimal staining was observed in the brains of the animals receiving Renographin and no staining was seen when Thorotrast was used Engorgement of the blood vessels particularly in the region of the basal ganglia was a common finding noted following the use of any of the media provided adequate amounts and concentrations were employed This effect was most marked in the brains of the animals receiving Diodrast and Urokon

The radiographic density of 50 per cent Hypaque compared favorably with that of Thorotrast Because of the necessity for using lesser concentrations of Diodrast Urokon, and Renographin the contrast densities of these media were considerably less than either Thorotrast or Hypaque

**Clinical Results** Percutaneous carotid angiography was performed on patients in all age groups with satisfactory visualization of the cerebrovascular system Figure 1 shows a preoperative angiogram of a patient in whom Thorotrast was used compared with a postoperative angiogram on the same patient using Hypaque

Most of the angiograms were done using 2 injections of 10 to 12 cc each of 50 per cent Hypaque for each side injected Occasionally, larger amounts were used for better visualization of a lesion Figure 2 illustrates angiograms using first 10 cc and then 20 cc to more clearly demonstrate the lesion

No complications have occurred from the use of these larger quantities of Hypaque The use of larger quantities of contrast medium lends itself to the employment of single exposure technique When the Fairchild camera or other multiple exposure techniques are used much smaller amounts give satisfactory studies

**Complications** All patients were skin tested with Hypaque prior to angiography and no responses contra indicated the use of the medium There were *no clinical manifestations of allergic responses to Hypaque This is in* agreement with observations made by Lowman *et al*[2] in their experience with Hypaque in excretory urography

The effects of the rapid injection of Hypaque into the carotid arteries of

gesia spotty areas of hypalgesia below the level of analgesia and recurrent pain related to analgesic areas

Ten patients have therefore been selected over the course of the last 3 yrs for study with electrical stimulation in an attempt to further define the changes in sensation which follow this procedure Only those patients who gave reproducible reports of threshold values for sensations were studied in detail

METHOD

In the early phase of this study skin stimulation was used A monopolar electrode with a 1 mm silver ball on the end of a coil spring was moved lightly and slowly over predetermined areas of normal skin In this manner the threshold values for touch or tingling and for pricking pain were outlined for the arm thorax abdomen, and leg Reproducible results could be obtained at 1 sitting but varied from day to day depending on the humidity perspiration and room temperature Frequencies were varied from 1 to 40 or 100/sec and voltage varied to threshold Duration of each square wave pulse varied from 0 02 to 2 msec Only touch and prick thresholds could be measured by this technique Therefore an insulated electrode with a grounded shield was inserted into the superficial radial nerve of 2 patients after cordotomy with total analgesia to C3 and C5 respectively Again stimuli of 0 02 to 2 msec duration and frequencies of 1 4 10 and 40/sec were used raising the voltage to threshold A pressure cuff was placed on the arm after touch thresholds were determined and thresholds for pricking pain and finally burning aching pain were determined

Drugs were given to some patients to determine their effect on these pain threshold studies Demerol and morphine and I V pentothal in dosage sufficient to cause drowsiness were given to 2 patients and 5 to 40 per cent nitrous oxide was given to 5 The threshold studies could be determined with ease and full cooperation at 20 per cent nitrous oxide Four patients were totally unaware of being given anything but oxygen until they were given 30 per cent nitrous oxide and were unreliable only near 40 per cent nitrous oxide

DISCUSSION

White and Sweet[1] in their recent classical work on pain state that 10 patients who were tested with electrical skin stimulation in analgesic areas after a clinically successful cordotomy all reported unpleasant or painful sensations In general we have confirmed their observations in our patients However in 1 instance the patient reported a sensation of a throb rather than pain when an electric stimulus strong enough to spark to her skin was applied while still in the operating room Six hours later however she reported pricking painful sensations on skin stimulation In 2 *instances* pain was referred to other areas once from a small analgesic area on the thorax to the hypalgesic ipsilateral axilla and once to a mirror *position on the* normal right side of the abdomen from a punctate area on the analgesic left side

Threshold values on the analgesic side exceeded in voltage the normal control side by only 2 to 6 times at a given frequency In 1 case *followed for* 18 mo the values on the side of analgesia were within the range of variability on the normal side

showed a complete occlusion of the basilar artery with atheromatous plaques. A third patient, a 34 yr old colored female, developed a unilateral facial weakness following angiography. The weakness disappeared after 2 days.

DISCUSSION

Angiographic studies obtained with the use of Hypaque as the contrast medium were of excellent contrast quality. The radiographic density is less than that obtained with Thorotrast, but superior to that obtained with safe concentrations of Diodrast or Urokon. Renographin appears to be a safe medium in lower concentrations, although some degree of contrast density is lost in dilution.

In a previous publication we reported angiographic complications using Diodrast or Thorotrast in 5 patients in 215 studies.[3] All complications occurred in patients over the age of 60 suspected of having cerebrovascular disease. Fewer complications have occurred with Hypaque. Smolik and Nash[4] reported a similar experience using Hypaque.

The discomfort to the patient during the injection of Hypaque under local anesthesia can be reduced either by the use of lesser concentrations of the medium, or by the use of smaller quantities per injection during multiple exposure technique.

SUMMARY

An experimental and clinical evaluation of Hypaque Sodium as a contrast medium for cerebral angiography shows that it is superior to other media presently in use. Its superiority is based upon its tolerance by the patient at the time of injection, a high degree of radiographic density, and its safety in patients over the age of 50.

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## POST CORDOTOMY STUDIES OF PAIN THRESHOLDS*

ROBERT B. KING

Spinothalamic tractotomy has become a well established neurosurgical procedure for the relief of intractable pain. However, reports indicate that from 10 to 30 per cent of these patients are either not totally or permanently relieved of pain by this procedure. These failures have shown a number of disturbing clinical findings including falling and fading levels of anal

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gesia spotty areas of hypalgesia below the level of analgesia and recurrent pain related to analgesic areas

Ten patients have therefore been selected over the course of the last 3 yrs for study with electrical stimulation in an attempt to further define the changes in sensation which follow this procedure Only those patients who gave reproducible reports of threshold values for sensations were studied in detail

METHOD

In the early phase of this study skin stimulation was used A monopolar electrode with a 1 mm silver ball on the end of a coil spring was moved lightly and slowly over predetermined areas of normal skin In this manner the threshold values for touch or tingling and for pricking pain were outlined for the arm thorax abdomen and leg Reproducible results could be obtained at 1 sitting but varied from day to day depending on the humidity perspiration and room temperature Frequencies were varied from 1 to 10 or 100/sec and voltage varied to threshold Duration of each square wave pulse varied from 0 02 to 2 msec Only touch and prick thresholds could be measured by this technique Therefore an insulated electrode with a grounded shield was inserted into the superficial radial nerve of 2 patients after cordotomy with total analgesia to C3 and C5 respectively Again stimuli of 0 02 to 2 msec duration and frequencies of 1 1 10 and 40/sec were used raising the voltage to threshold A pressure cuff was placed on the arm after touch thresholds were determined and thresholds for pricking pain and finally burning aching pain were determined

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# DECREASE OF SPASTICITY BY PHYSIOLOGICAL DORSAL ROOT RHIZOTOMY*

G M AUSTIN G P McCOUCH AND F C GRANT

Previous work on the spinal cord of cats and monkeys showed that it was possible to identify and measure the potential derived from afferent terminals ($N_1a$) within the spinal cord (Fig 1) when a dorsal root was stimulated[1] One of us (G McC) had previously conceived the idea that spasticity might result from sprouting of new dorsal roof afferent terminals within the spinal cord (Fig 2) Utilizing the potential of afferent terminals ($N_1a$) as a tool we attempted to determine whether or not this was the case in chronically hemisected cats and monkeys In chronic midthoracic hemisections of the spinal cord where a spastic monoplegia was produced $N_1a$ was increased in amplitude from 10 to 100 per cent on the side of the monoplegia (Fig 3) In addition histologic studies showed that there was an increase of 8 to 58

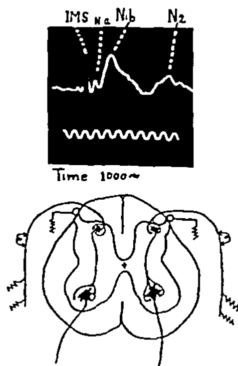



Fig 1 Bottom Shows diagram of spinal cord at site of stimulation and recording Top Cathode ray photo of cord potential recording response to stimulation of L6 dorsal root from the root entrance zone

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Stimulation of the superficial radial nerve using a pressure cuff so as to be able to study C fiber pain gave further findings in 1 case with clinical analgesia to C2. Threshold values were similar to a normal control subject and the patient clearly gave thresholds for tingling, pricking pain, and burning pain. On 1 occasion she complained bitterly of a deep aching pain at the top of the pressure cuff after 31 min and stimulation at this time for C fibers caused severe burning pain, sweating, nausea, and great anguish.

When 20 per cent nitrous oxide was given to these patients 2 observations of note were apparent. In 5 instances where there had been a distinct drop in their level of analgesia to pin prick, the level rose quickly to its highest postoperative level. This observation was made in 1 instance 18 mo after unilateral high cervical cordotomy in which the level had fallen from C5 to T8 and with 20 per cent nitrous oxide again rose to C5 within 12 min. Despite this dramatic rise in the level of pin prick analgesia there were only minor changes of threshold for prick or electrical skin stimulation. In 2 instances where the analgesic level fell far enough to allow a return of spontaneous pain the patients stated under 20 per cent nitrous oxide their spontaneous pain also disappeared with the ascent of the pin prick analgesia.

Stimulation of the superficial radial nerve under pressure block and 20 per cent nitrous oxide in 1 case showed no evidence of summation at high frequency stimulation of C fibers but thresholds were otherwise not altered. Without the 20 per cent nitrous oxide this patient showed clear evidence of summation in C fiber responses in that threshold values for pain fell approximately 60 per cent at 400/sec but were not measurably different for this range of frequencies with the nitrous oxide.

These observations suggest 2 possible explanations. It may be that an injured or partially sectioned spinothalamic pathway allows passage of well synchronized electrical stimuli and to a lesser degree the relatively unsynchronized impulses generated by pin prick from hypalgesic skin areas. In such a circumstance nitrous oxide may interfere with conduction at the region of trauma enough to block the passage of pin prick stimuli but not those due to brief electrical stimulation. An alternate explanation suggests that a pain pathway exists in man aside from the spinothalamic system. It is known that there is no direct spinothalamic system in cats and dogs and very little in monkeys. Even in lower primates Marchi degeneration studies show few fibers entering the thalamus and in cats no apparent sensory deficit follows simple section of the anterolateral column of the spinal cord. In these lower forms there is apparently a multisynaptic system or small unmyelinated fiber system for conduction of pain impulses which is dispersed widely through the cord and may even cross from left to right and back across the spinal cord as suggested by the observations of Karplus and Kreidl[2] cited by White and Sweet.

The observations on these 10 patients suggest that man may have a system in the spinal cord for relaying a painful stimulus to higher centers in the absence of spinothalamic conduction. The effectiveness of small amounts of nitrous oxide in blocking this system suggests a multisynaptic chain or system and the facility with which highly synchronized electrical impulses may be transmitted across this pathway without the passage of relatively poorly synchronized stimuli (as a pin prick or scratch) strengthens such a concept.

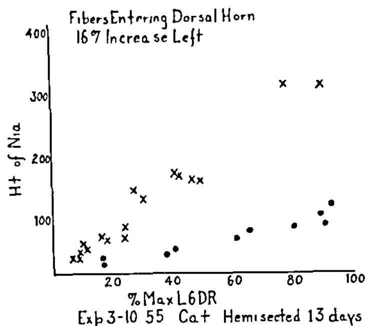



Fig 3 Graph plotting amplitude of $N_1$ a potential against percent of maximal dorsal root spike height The crosses refer to the chronically hemisected side and the dots to the control side (acutely hemisected)

tive procedure for permanently stopping spasticity in total paraplegia[4] However when there is only partial spinal cord section with resulting para paresis and spasticity selective dorsal rhizotomy may prove extremely effec tive When remaining downstream fibers have regenerated beyond the site of injury and reach their former destination in the interneuronal pools in volved in motor movement there is unavailable space left for them to re establish synaptic endings One way of permitting them to re establish synaptic connections is to temporarily block and cause degeneration of the dorsal root afferents which have proliferated and assumed control over spinal cord function Thus a selective dorsal root rhizotomy would decrease spasticity and provide the mechanism by which downstream fibers if they have regenerated past the site of injury may return to the point of pro ducing effective motor movement The ultimate result of selective dorsal root rhizotomy probably depends on a competitive sprouting between re maining adjacent dorsal roots and corticospinal fibers

We have now operated on 6 patients who have shown severe spasticity with various degrees of mass reflexes It was with the anticipation that the procedure would decrease the severe flexor spasms and mass reflexes that the operation was carried out However 3 of these patients had slight movement of the leg foot and toes It was felt that if their downstream afferents were intact the operative procedure itself would provide optimal conditions for improved motor function Obvious difficulties are apparent in this line of attack First one is unsure as to how much remains or has regenerated in the way of downstream pathways past the site of injury Another and more serious problem in this type of procedure is that unless patients are seen fairly early in the course of their disease one will encounter severe contrac tures of the adductors hamstrings and achilles tendons Then the proce dure although it may be markedly helpful faces the physical opposition of the tendon contractures Finally an extremely important factor has proven to be intelligent motivation Unless a patient is willing to intensively prac tice to improve motor movement the operation is unlikely to be successful

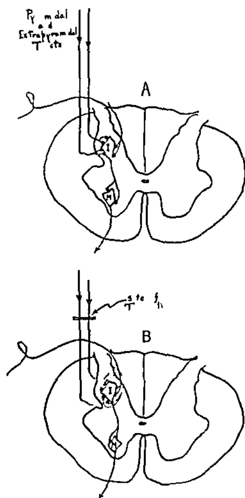



Fig. 2. A. Normal relationship of downstream pathways and dorsal root fibers ending on interneurons. B. Sprouting of new dorsal root afferents following cord injury.

per cent in the number of afferent fibers from the dorsal root entering the dorsal horn on the side of the chronic hemisection at the site of stimulation. This provided evidence for a hypothesis of the development of spasticity. If the spinal cord is damaged severely enough to interrupt downstream axons these fibers degenerate from the boutons upward toward the site of injury. Then within a period of 1 to 2 wks. there is a gradual sprouting of new afferent terminals from dorsal roots. These terminals end predominantly on interneuronal pools and form synaptic connections in positions left vacant by degenerated downstream terminals. Once synapses have been established by the newly sprouted afferents the effective stimulus over these dorsal roots is greatly increased from the normal situation. This provides the necessary mechanism for hyperactivity of the reflexes following damage to downstream pyramidal and extrapyramidal systems.

With this physiologic background in mind it seemed that one way to decrease spasticity and pain associated with mass reflexes in the human would be to limit the afferent input to the interneuronal pools. This is best done by interruption of selected dorsal roots and their freshly sprouted afferents (Fig. 2). However, if a few dorsal root afferents are cut there are always other dorsal root afferents capable of sprouting from adjacent levels and which according to the evidence of Liu and Chambers[3] may cover a distance of as much as 6 segments in their attempts to establish synaptic connections. This suggests why rhizotomy of only 3 or 4 dorsal roots remains an ineffec

# CLINICAL AND EXPERIMENTAL STUDIES ASSOCIATED WITH ELECTROLYSIS OF LISSAUER'S TRACT*

Robert W Rand Ernest J Penka and W Eugene Stern

In 1912 and 1913 Hyndman and coworkers[1,2] focused attention on Lissauer s tract in man by demonstrating that its destruction in the thoracic cord resulted in ipsilateral analgesia and thermanesthesia in a band 3 to 5 segments wide Such procedures in the cervical cord did not give similar results Stimulated by the potentialities of these results a program employing a technique of electrolysis of this and other spinal cord tracts was commenced[3]

## METHOD

Five cats and 2 monkeys underwent laminectomies using general nembutal anesthesia and sterile conditions Neither wound nor meningeal infection occurred In this first series of experiments unipolar direct low amperage current (0 5 to 1 0 ma) needle electrolysis for 15 to 45 sec. was employed in different combinations to produce multiple lesions in Lissauer s tract In the postoperative period neurological examinations and cinematography assessed the neurologic changes The cervical and upper thoracic cords were serially sectioned and stained alternately by hematoxylineosin and Smith s myelin methods 3 wks to 3 mo after the Lissauer tractolysis

In addition multiple lesions in Lissauer s tract have been made during the course of thoracic cord surgery on 2 cancer patients using 0 5 ma for 30 sec for each of 6 and 9 lesions respectively

## RESULTS

**Cat 1** Three lesions were produced on the left side at 1 mm depths in the Lissauer tract of the C6 and C7 cord segments using 1 0 ma for 30 sec at C8 and T1 levels there were similar lesions with 0 5 ma for 15 sec. On the right 3 lesions were made in this tract in the cord segment C6 7 8 and T1 using 0 5 ma for 30 sec Immediately after operation analgesia was present in the left forearm and paw with marked hypalgesia on the right in similar areas The cat could move all extremities but was unable to walk After 3 wks the animal could walk in a slinking manner with truncal awkwardness Seven weeks postoperatively almost normal climbing and running was possible There was marked hypalgesia of the left forearm and paw and moderate hypalgesia of the right Motor function improved steadily but the sensory deficit persisted No motor impairment was noted when sacrificed 3 mo after operation

A wedge shaped scar replaced the entire Lissauer tract over the C6 segment on the left and extended slightly into the upper dorsal spinocerebellar tract and the posterior column On the right small intermittentently spaced areas of gliosis were noted in Lissauer s tract There were 2 small areas of myelomalacia in the base of each posterior horn Similar findings were noted in the C7 cord segment No distinct lesions in Lissauer s tract were found at the

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Our initial 3 cases were of paraplegia following spinal transection Extensive dorsal rhizotomy (L1–S1) relieved the pain associated with the spasms but some of the spasticity returned in 3 to 6 mo Our fourth patient (K S) was completely blind following a tubercular arachnoiditis He had severe para paresis hamstring contractures with pain and spasticity An extensive dorsal rhizotomy (L2–S1) relieved a great deal of the spasticity and also his pain but he regained only slight motor power in the feet and toes Another pa tient (A T) had sustained a severe head injury with resulting spastic para paresis and weakness of the right arm In addition there was mental impair ment following the injury Dorsal rhizotomy was again ineffective (L3 L5 S1) in producing more than 15 per cent motor recovery in the feet and toes His spasticity was decreased temporarily but he was never able to overcome the tendon contractures of the hamstrings and seemed to possess no strong motivation to improve

Our sixth case was that of a 30 yr old white woman with marked para paresis severe extensor and adductor spasms but no tendon contractures She had been shot at T10 with resulting cord contusion Prior to operation she was unable to stand except when supported by 2 people Dorsal rhizot omy of L2 L4 and S1 was done bilaterally, 6 mo after the injury Six months after the rhizotomy she can walk unassisted with the use of a cane Her extensor spasms were stopped and motor power continues to increase She has a great deal of motivation and has exercised faithfully in attempts to help herself

In conclusion selective dorsal rhizotomy seems worthwhile in certain cases of paraparesis when spasticity is an incapacitating factor and where lack of tendon contractures and a strongly motivated patient favor recovery

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ficial Lissauer tract lesions associated with localized arachnoiditis were slightly misplaced medially in the lowest cord segments Varying degrees of myelomalacia was observed throughout most of the transverse gray similar to Cat 1 The lateral aspect of the anterior horns was involved in some segments as well as the base of the posterior columns

The weakness exhibited in the right paw was certainly due to anterior horn cell involvement The hypalgesia was undoubtedly the result of myelomalacia of the transverse gray and the base of the posterior horns rather than lesions in Lissauer's tract The infarctions were strikingly similar to Cat 3 and 4 where there was also a mild subarachnoid hemorrhage along a posterior root

**Monkey 1** Ten lesions were produced 1 mm deep in Lissauer's tract on the right with 0 5 ma for 15 sec from the sixth cervical through the first thoracic cord segments The gross and fine movements and coordination of the extremities during climbing jumping and eating were normal postoperatively except for some initial swaying of the pelvis for 1 wk Many attempts to test sensory dysfunction failed However while undergoing slow anesthesia prior to necropsy examination 6 wks after operation a definite analgesia of the entire ulnar aspect of the right forearm and a profound hypalgesia in the ulnar distribution of the right hand and fourth and fifth fingers were observed In contrast quick violent retraction of the other extremities occurred during forceful painful stimulation From the mid portion of C6 through the upper T1 cord segments the right Lissauer tract was replaced at the site of the lesions by either a dense wedge shaped cicatrix extending to the substancia gelatinosa or lytic area conforming to the Lissauer tract (Fig 1) Although quite limited to the Lissauer area in C6 7 and T1 levels it spread slightly into the posterior column and the upper portion of the dorsal spinocerebellar tract at the C8 cord segment There was an area of myelomalacia in the base of the left posterior horn at T1 levels At the T2–3 cord levels myelomalacia appeared in the transverse gray In places this malacia extended slightly into the left posterior column and the lateral funiculi

The analgesia and hypalgesia of the right forearm and hand were undoubtedly due to interruption of the right Lissauer tract No other pathological changes from C6 through T1 cord segments were present The central myelomalacia of T2–3 levels was well below the site of the last lesion and was not associated with significant arachnoiditis This malacia probably did not contribute to the neurologic sensory deficit of the right upper extremity

**Monkey 2** Fifteen lesions 1 mm deep were made in the right Lissauer tract from C5 through T1 cord segments with 0 5 ma for 30 sec Postopera



Fig 1 Electrolytic lesion in the right Lissauer tract of monkey 1 causing its complete destruction at the C8 T1 level without other significant pathological changes

C8 level Initially myelomalacia at the base of the left posterior horn extended into upper portions of the anterior horn and adjacent white substance Thereafter it spread to involve the transverse gray In the T1 cord segment several superficial lesions in the Lissauer tract were present Similar degenerative changes in the entire transverse gray continued including the lateral funiculus medially

In spite of early motor disturbances this animal eventually ran climbed and jumped normally Apparently the hypalgesia in the forearms and paws resulted from a combination of involvement of the transverse gray posterior horn and Lissauer s tract

**Cat 2** Three lesions each 3/4 mm in depth were made in the C5 6, 7 8 and T1 cord segments employing 1 0 ma for 30 sec The animal died of nembutal intoxication Some of the lesions were found to be misplaced lateral to the Lissauer tract Thereafter magnifying glasses resulted in greater accuracy of needle placement

**Cat 3** Using 0 5 ma for 30 sec at a depth of 1 mm a total of 15 lesions were produced along Lissauer s tract from C5 through T1 cord segments on the right A small subarachnoid hemorrhage occurred at the left C6 level Although there was poverty of movement of the right forearm, and the animal walked in a stalking manner for 2 wks thereafter it ran jumped and climbed without any apparent paresis or loss of position sense During this period analgesia of the entire right upper extremity and moderate hypalgesia of the left to the elbow was noted The right sided analgesia gradually changed to a marked hypalgesia while normal sensation returned on the left Incomplete lesions along Lissauer s tract associated with the usual amount of arachnoiditis were found 6 wks after operation Varying degrees of myelomalacia of a considerable portion of the transverse gray at each cord segment was observed The anterior commissure was not completely involved even in the areas of most marked myelomalacia In a few areas demyelinization of the lateral funiculus was present medially

One would have expected the bilateral hypalgesia to be persistent if the transverse gray myelomalacia were totally responsible Therefore it is suggestive that the Lissauer tract lesions even though superficial contributed to the right upper extremity hypalgesia

**Cat 4** This animal is included as a control A small local subarachnoid hemorrhage over the right C6 root occurred during the exposure as it had in Cat 3 No Lissauer tract lesions were made The animal developed marked paresis of the right forepaw and bilateral analgesia to the elbows The symptoms persisted in lessened degree over the 3 wk survival period An area of severe myelomalacia at C6–7 involved the lateral portions of the right anterior horn as well as the base of the right posterior horn and the anterior commissure in some areas

**Cat 5** Five left Lissauer tract lesions were made 0 5 mm deep in the cord segments C5 6 and 7 four in C8 and 3 in T1 employing 0 5 ma for 15 sec A small subarachnoid hemorrhage occurred at the right C6–7 level There was marked paresis of the right paw and questionable hypalgesia which disappeared in 3 wks permitting normal motor activity Although difficult to test and evaluate at first due to uncooperation adequate examination later disclosed definite hypalgesia of the left forearm and paw being most marked distally which persisted to time of autopsy 1 mo after surgery The super

ficial Lissauer tract lesions associated with localized arachnoiditis were slightly misplaced medially in the lowest cord segments Varying degrees of myelomalacia was observed throughout most of the transverse gray similar to Cat 1 The lateral aspect of the anterior horns was involved in some segments as well as the base of the posterior columns

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Fig 1 Electrolytic lesion in the right Lissauer tract of monkey 1 causing its complete destruction at the C8 T1 level without other significant pathological changes

tively the animal had severe weakness of the forearms and hands especially on the right This improved slightly Sensation to pain was appreciated in the left upper extremity and hand and in the right thumb and index finger Analgesia of the ulnar aspect of the right hand and forearm was present A severe paraparesis in extension progressed over the course of 2 wks into a complete paraplegia in flexion below the T2 cord segment The sensory findings in the upper extremities remained essentially unchanged at the time of autopsy 3 wks after operation Multiple infarctions of the right Lissauer tract from C6 through C8–T1 levels extended into the lateral half of the ipsilateral transverse gray and upper portions of the anterior horn Below T1–2 levels the entire central gray was completely destroyed with demyelinization extending well into the lateral funiculi

Rather than cicatrix lesions of the Lissauer tract these lesions were clearly vascular in type but apparently did result in the analgesia over the C7 through T1 dermatomes on the right Below T1–2 levels the severe central infarction caused the eventual complete paraplegia in flexion

**Patient 1** Nine lesions were placed in the right Lissauer tract over the T2 3 and 4th cord segments at a 1 mm depth using 0 5 ma current for 30 sec No areas of hypalgesia developed There was no paresis of the legs Five days later posterior rhizotomy of T2 through T5 was carried out to relieve the intercostal pain secondary to brochogenic carcinoma

**Patient 2** Six lesions were made in the right Lissauer tract at T3–4 levels during the course of a right anterolateral cordotomy at T2 for the relief of unilateral left leg pain due to metastatic carcinoma invasion of the left lumbo sacral plexus The lesions were 1 5 mm deep and produced by 0 5 ma for 30 sec Again no sensory changes occurred attributable to the Lissauer tract lesions Neither weakness of the right or left leg nor sensory disturbance of the right lower extremity was observed

## SUMMARY AND CONCLUSIONS

The electrolytic technique herein described can produce discrete multiple lesions in Lissauer s tract

In the absence of other associated spinal cord changes the resultant analgesia and hypalgesia is probably related to these lesions in Lissauer s tract (monkey 1)

Associated distant vascular myelomalacia can occur with this technique due either to subarachnoid hemorrhage from tearing of a small posterior radicular artery or electrocoagulation of penetrating vessels in the posterior root

The application of this electrolytic technique had as yet not been associated with persistent analgesia in man

Further modification of this electrolytic technique is being undertaken in an effort to avoid distant vascular infarction in the spinal cord

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# SELECTIVE SACRAL NEURECTOMY FOR HUNNER'S ULCER WITH NEUROPHYSIOLOGIC OBSERVATIONS*

Robert D Whitfield Paul W Myers William A Milner and Frederick Campbell†

The treatment of chronic interstitial cystitis has been difficult and discouraging since Hunner first described the condition in 1911 Over the years it became a clinical entity sometimes unrecognized because of the obscurity of the pathology Hunner in fact called the condition elusive ulcer Today the disease is known as chronic interstitial cystitis or Hunner s ulcer

Over 95 per cent of the cases occur in females who complain of frequency and pain on urination usually occurring in irregular cycles Cystoscopic examination shows linear cracks in the bladder mucosa at the dome that bleed on the slightest provocation Surrounding this cracked mucosa is a radiating network of congested vessels

Meredith Campbell in his text of urology states no other condition except cancer or tuberculosis of the bladder can cause such intractable or severe pain As in any disease of obscure etiology treatment is varied

Hunner first recommended partial suprapubic cystectomy This radical procedure has been followed by bladder distention silver nitrate irrigations radium vitamin E estrogens presacral neurectomy and frequent fulguration It was clear that any approach to permanent relief of pain and frequency for these unfortunate individuals lay in a new direction

Meirowsky[1] first performed selective sacral neurectomy in 2 patients for the treatment of Hunner s ulcer Personal communication with him prior to the publication of his results aroused our interest in the problem

Our series consists of 6 patients who were studied and operated upon between December 1955 and May 1956 Five of these had typical Hunner s ulcers All of them were female ranging in age from 35 to 69 with an average age of 53 2 years and a median age of 52 years Their symptoms had been present between 5 and 12 yr with an average of 9 2 yr and a median of 8 5 yr All had severe pain All had had fulguration silver nitrate instillation dilatation of the bladder and sulfonamides or various combinations of these Two had had gross hematuria 1 microscopic 2 none In every instance the ulcer had been observed cystoscopically

The sixth was a 51 year old male who had transurethral resection of a carcinoma of the bladder 3½ yr previously An ulcer resulted which had many of the characteristics of Hunner s ulcer—appearance severe pain frequency of urination and gross hematuria For these reasons he was considered a suitable candidate Unfortunately massive recurrence of his primary tumor prevented any lasting benefit Nonetheless the observations made before operation contribute their part to this study and are therefore included

## METHOD

**Preoperative Observations** Prior to operation selective blocks of the sacral nerves were made under radiographic control Bladder capacity was

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†Deceased

*Table 1 Observations at the Time of Block*

| PATIENT | NERVES BLOCKED | BLADDER CAPACITY (CUBIC CENTIMETERS) BEFORE | AFTER | BLADDER PRESSURE mm $H_2O$ BEFORE | AFTER |
|---|---|---|---|---|---|
| 1 (B H) | S3 | 45 | 170 | NOT MEASURED | |
| 2 (H I) | S3 | 30 | 200 | 600 | 130 |
| 3 (M I) | S3 | 175 | 200 | 320 | 210 |
| 4 (C A) | S3 | 290 | 380 | 170 | 160 |
| 5 (R S) | S3 | 25 | 60 | 210 | 220 |
| 6 (M R) | S3 | 30 | 50 | 360 | 100 |
| | S2 | 50 | 60 | 350 | 580 |

determined before and after block and, the intravesical pressure at maximum distention was also established The results are recorded in Table 1

**Operative Exposure** Midline incision was made over the sacrum The musculature was stripped back beyond the sacral crests bilaterally The sacral foramina were identified by reference to the spinous process of the 5th lumbar vertebra The second and third sacral foramina were unroofed the nerves (anterior primary rami) freed and, in 3 cases the second and third sacral nerves stimulated in turn while cystometrographic observations were made A member of this group estimated by digital examination the contractions of the anal sphincter and of the levatores ani The third sacral nerves were then sectioned in 5 cases the second sacral nerves were ligated and the distal segment injected with 95 per cent alcohol in Case #6

**Observations at Operation** In the later cases 3 in number the results of stimulation were recorded (Table 2) In 1 instance the second and third sacral nerves contributed to bladder innervation in the other 2 the third sacral nerve alone was of importance

RESULTS

As shown in Tables 3 and 4 material subjective and objective improvement was secured while the undesirable effects were slight The relief of pain and decrease in frequency of urination were striking Four ulcers healed 1 has continued to have recurrent microscopic hematuria Case #6 who had recurrent carcinoma is excluded

*Table 2 Observations at Operation*

| PATIENT | NERVES STIMULATED BILATERALLY | CHANGE IN BLADDER PRESSURE (mm $H_2O$) | RECTAL SPHINCTER SPASM | LEVATOR ANI CONTRACTION | NERVES DIVIDED BILATERALLY |
|---|---|---|---|---|---|
| 2 (H I) | S2 | 320 150 | Marked | | |
| | S3 | 320 180 | Slight | | S3 |
| 4 (C A) | S2 | None | Marked | None | |
| | S3 | 200 600 | None | Moderate | S3 |
| 5 (R S) | S2 | None | None | Moderate | |
| | S3 | 210 600+ | Marked | None | S3 |
| No stimulation in Cases #1, 3 and 6 | | | | | |

*Table 3 Post Operative Results (Bladder)*

| PATIENT | NERVES DIVIDED | BLADDER CAPACITY (CUBIC CENTIMETERS) PREOP | POSTOP | FREQUENCY PREOP | POSTOP | HEALING OF ULCER |
|---|---|---|---|---|---|---|
| 1 (B.H.) | S3 | 45 | 180 | 10 min | 4 hr | Yes |
| 2 (H.T.) | S3 | 50 | 300 | 20 min | 3 hr | Yes |
| 3 (M.P.) | S3 | 1[illegible] | [illegible]00 | 1[illegible] min | 3 hr | Uncertain |
| 4 (C.A.) | S3 | 200 | 300 | 1 hr | [illegible] hr | Yes |
| (R.S.) | S3 | – | – | 10 min | 2[illegible] hr | Yes |

Note Patient #6 (M.R.) had recurrence of carcinoma requiring total cystectomy

*Table 4 Post Operative Deficits*

| PATIENT | RECTAL SPHINCTER TONE—% OF NORMAL | RECTAL INCONTINENCE | NEUROLOGIC DEFICIT |
|---|---|---|---|
| 1 (B.H.) | 75% | None | None |
| 2 (H.T.) | 75% | None | Hypalgesia lateral left thigh |
| 3 (M.P.) | 75% | None | Hypalgesia S3 absent gluteal reflexes |
| 4 (C.A.) | 50% | None | Perineal numbness (subjective) |
| (R.S.) | 75% | None | Absent ankle jerks |
| 6 (M.R.) | 7[illegible]% | None | Hypalgesia S2 S3 |

Recurrent carcinoma

SUMMARY

1 Observations upon 5 patients suffering from typical Hunner's ulcers and upon 1 with a similar ulcer resulting from carcinoma of the bladder are recorded

2 In all our cases the third sacral nerves contributed to bladder detrusor activity

3 In 2 cases the second sacral nerves did not contribute to bladder detrusor activity in 2 they did and in 2 our observations do not provide any information

4 In all 5 cases of true Hunner's ulcer bilateral section of the third sacral nerves produced subjective relief of symptoms and objective evidence of improvement

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*Table 1 Observations at the Time of Block*

| Patient | Nerves Blocked | Bladder Capacity (cubic centimeters) Before | After | Bladder Pressure mm $H_2O$ Before | After |
|---|---|---|---|---|---|
| 1 (B H) | S3 | 45 | 170 | Not measured | |
| 2 (H I) | S3 | 30 | 200 | 600 | 430 |
| 3 (M I) | S3 | 175 | 200 | 320 | 210 |
| 4 (C A) | S3 | 290 | 380 | 170 | 160 |
| 5 (R S) | S3 | 25 | 60 | 210 | 220 |
| 6 (M R) | S3 | 30 | 50 | 360 | 400 |
| | S2 | 50 | 60 | 350 | 580 |

determined before and after block and the intravesical pressure at maximum distention was also established The results are recorded in Table 1

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**Observations at Operation** In the later cases, 3 in number the results of stimulation were recorded (Table 2) In 1 instance the second and third sacral nerves contributed to bladder innervation in the other 2 the third sacral nerve alone was of importance

RESULTS

As shown in Tables 3 and 4 material subjective and objective improvement was secured while the undesirable effects were slight The relief of pain and decrease in frequency of urination were striking Four ulcers healed, 1 has continued to have recurrent microscopic hematuria Case #6 who had recurrent carcinoma is excluded

*Table 2 Observations at Operation*

| Patient | Nerves Stimulated Bilaterally | Change in Bladder Pressure (mm $H_2O$) | Rectal Sphincter Spasm | Levator Ani Contraction | Nerves Divided Bilaterally |
|---|---|---|---|---|---|
| 2 (H I) | S2 | 320 150 | Marked | | |
| | S3 | 320 380 | Slight | | S3 |
| 4 (C A) | S2 | None | Marked | None | |
| | S3 | [illegible] | None | Moderate | S3 |
| 5 (R S) | S2 | None | None | Moderate | |
| | S3 | 210 600+ | Marked | None | S3 |

No stimulation in Cases #1 3 and 6





Figs 1 2 and 3 amplify the text After introduction into the nerve ends atraumatic sutures are threaded on a long needle for passage through the tube and are then removed



strength is poor and experience has shown that a reinforcing matrix is advantageous Impregnation of the filter material on #58 stainless steel mesh overcomes the problem of fragility but sacrifices flexibility

Between the third and fourth week after implantation the unreinforced tubes were contained within gossamer thin sheathes of opalescent tissue which were loosely adherent to the tissue bed A slightly denser fibrous coat surrounded the reinforced tubes Within the tubes a nonadherent continu

# THE SURGICAL APPLICATION OF MONOMOLECULAR FILTERS (MILLIPORE) TO BRIDGE GAPS IN PERIPHERAL NERVES AND TO PREVENT NEUROMA FORMATION*

JAMES B. CAMPBELL AND C. ANDREW L. BASSETT

The problem of inducing orderly regeneration across an irreducible gap in a peripheral nerve has been approached by grafting,[1] anastomosis following staged lengthening,[2] insertion of a filament scaffold within a vein graft,[3] and securing of the proximal and distal stumps within tubes.[4] The reported limitations of these techniques prompted a search for an ideal conduit within which continuity could be restored by regeneration. Such a conduit should *be inert in tissues, permit free passage of extracellular nutrient fluids and* at the same time deny access to cells. When the *in vivo* properties of a monomolecular cellulose filter (Millipore H.A. formulation) were announced by Algire,[5] these qualifications appeared to be fulfilled.

## METHOD

Initially, sheets of moistened Millipore were rolled over a glass rod into tubes 6 cm. in length and 3 to 4 mm. in diameter. This form was maintained by a narrow line of bonding with Celvai or Glyptal. The process could be likened to fabricating a cigarette without tobacco. However, the incidence *of rupture of tubes made reinforcement of the filter material desirable.* Therefore, cylinders were fabricated from #58 stainless steel mesh sheets impregnated with Millipore material.

The tibial division of the feline sciatic nerve was isolated and severed. In 1 series of cats, the proximal and distal nerve ends were introduced into tubes of Millipore for a distance of 1 cm. A gap of 2.5 cm. in the nerve was maintained by suturing the proximal and distal segments to the muscle bed or to the tube (Fig. 1, 2, 3). In the initial phases of the experiment, a sling stitch between the proximal and distal segments prevented their withdrawal *from the tube. In a second group of animals, the proximal and distal nerve* segments were introduced into closely fitted blind end socks of Millipore. Because of discrepancies in the diameter of the tubes and nerves, tube ends were crimped with silver clips to create a snug fit and prevent ingress of scar tissue. Between the third and fourth postoperative week, the filter was removed and the nerve excised for histological study. However, in 1 cat in which the gap had been satisfactorily bridged at 4 wks., the reunited nerve was returned to its tissue bed for another 6 wks. after removal of the filter. In a control series, the surgery was identical except that Millipore tubes or socks were not applied.

## RESULTS

Millipore is brittle. Saturation with water increases flexibility to the extent that *careful manipulation becomes possible. However, at best, the tensile*

* From the Departments of Neurological and Orthopedic Surgery, Columbia University College of Physicians and Surgeons, and The New York Orthopaedic Hospital, Columbia-Presbyterian Medical Center, New York, New York. Supported by grants from the Department of the Army, Office of the Surgeon General (Contract No. DA 49 007 MD 515), Playtex Park Research Institute, and United Cerebral Palsy Associations, Inc.

Millipore supplied by Mr. John Bush, President, Millipore Filter Corporation, Watertown 72, Massachusetts.

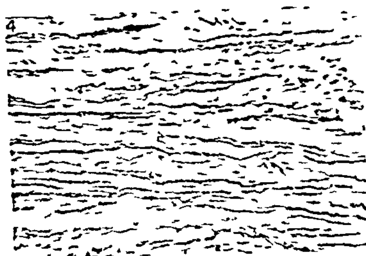



Fig 4 Bodian (×400) Longitudinal section of the distal nerve segment at 4 wks

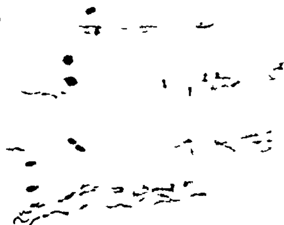



Fig 5 Mahon (×900) Longitudinal section of 2.5 cm tissue bridge at 10 wks

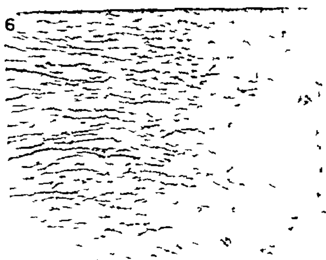



Fig 6 Bodian (×100) Longitudinal section of proximal nerve stump encased in a filter sock for 4 wks

From these data it seems that during the initial phases of regeneration cells from the circumference of the proximal nerve migrate distally using the walls of the tube as a substrate In most soft tissues nutrition by diffusion can occur over a distance of 1 mm It is therefore entirely possible that a layer of neural tissue 1 mm thick can build up on the inner wall of the tube completely independent of blood supply merely by drawing

ous firm opaque cord of tissue united the proximal and distal segments A striking contrast was provided by the control group and those animals in which the tubes were ruptured In both instances heavily vascularized bulbous neuromata had formed which were densely adherent to the tissue bed No tendency to bridge was noted in the gaps

In the 3 to 4 wk specimens there was histologic evidence of axons enveloped in parallel Schwann tubules extending the entire length of the 2 5 cm gap There was no evidence of myelin formation The longitudinal parallelism of fibers in the tissue bridge was a striking feature The sole evidence of disorganization was found about the sling stitches where a Schwannoma foreign body reaction prevailed However in tissues adjacent to the filter material no foreign body response had been evoked Study of serial longitudinal sections gave evidence that tissue at the periphery of the regenerated cord contained greater numbers of axons than did the central areas At 3 to 4 wks it was noted that the greater the distance from the proximal stump the fewer the axons In spite of these observations fibers had spanned the 2 5 cm gap and were observed entering the distal segment of nerve

A firm cord of tissue united the nerve segments in the animal in which the unsheathed bridge was returned to its bed 4 wks after the initial procedure At operation 6 wks later the bridge was easily dissected free by dividing a thin overlying film of tissue The stimulation of transection caused vigorous contractions of the gastrocnemius muscle as the major fascicles were cut 2 cm distal to the original lower level of sectioning The color of the cross section and fascicle diameters of the distal nerve were normal Mature axons in uniform numbers were found throughout the area of bridging and could be traced into the distal stump (Fig 4) Recanalization of the distal Schwann tubules by axons extended throughout the entire distance of the section Mahon stains indicated the presence of large quantities of myelin throughout the area of the gap (Fig 5) In the distal segment fewer myelin deposits were found

Covering of proximal and distal stumps with blind Millipore socks prevented neuroma formation At 4 wks after operation the Millipore material again had evoked a minimal host response Within the filter sock a firm opaque nonadherent nerve end was found its contours accurately reflecting the inner topography of its prison Microscopic examination of stained sections again demonstrated orderly, parallel axons projecting from the severed proximal nerve end (Fig 6) Schwann cell proliferation was not as prevalent as in the section taken from the nerve gaps Continuity was found to have been interrupted in two defective blind end socks In areas where rents had occurred in the Millipore material invading connective tissue elements created a picture typical of neuroma formation

DISCUSSION

The H A formulation of monomolecular cellulose filter material can be thought of in simplified terms as a flattened honeycomb with 1 mu openings The foregoing data suggest that the porocity of this particular plastic creates a favorable nutritional and substrate environment for peripheral nerve regeneration The material is most inert in tissues affords a scaffold for regenerating neural elements and shields them from competition with mesenchemal cells arising from the operative bed

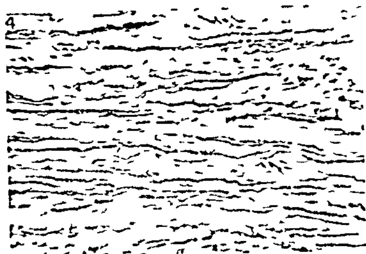



Fig 4 Bodian (X400) Longitudinal section of the distal nerve segment at 4 wks

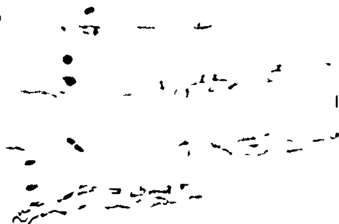



Fig 5 Mahon (X900) Longitudinal section of 2.5 cm tissue bridge at 10 wks

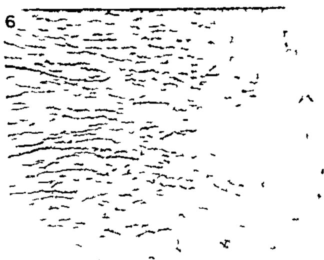



Fig 6 Bodian (X100) Longitudinal section of proximal nerve stump encased in a filter sock for 4 wks

From these data it seems that during the initial phases of regeneration cells from the circumference of the proximal nerve migrate distally using the walls of the tube as a substrate. In most soft tissues nutrition by diffusion can occur over a distance of 1 mm. It is therefore entirely possible that a layer of neural tissue 1 mm thick can build up on the inner wall of the tube completely independent of blood supply merely by drawing

nutrition from diffusing tissue fluids These factors probably account for the picture of greater tissue maturity seen at the circumference of the bridge at 3 to 4 wks In seeking reasons for the striking orderly parallelism of the regenerative picture it is interesting to speculate on the possible inductive role of the tube wall

Garrity[4] was able to limit neuroma formation by placing the severed ends of peripheral nerve within plastic and rubber tubes, but was unable to achieve regeneration The fact that mechanical rupture of the walls of the tubes or blind socks was invariably associated with neuroma formation emphasizes the importance of shielding the regenerating axons from outside cells The Schwannoma like formation in the region of the silk is provisionally accounted for on the basis of foreign body irritation In more recent experiments the sling stitch has been discarded Fixation sutures passing through the perineurium proximal and distal portions of the tubes have afforded adequate stabilization

The limited data indicate that the rate of regeneration is consistent with the general concepts of others Further experimentation utilizing bioelectric techniques will be necessary before definite statements can be made concerning rate of regeneration The preliminary findings of these studies demand that further work determine the tissue toxicity of Millipore and the functional adequacy of neural regeneration in long term animals Additional effort must be directed to the technical aspects of tube and sock production Millipore filter material unreinforced is brittle when dry friable when wet and generally difficult to handle Clinical application of the technique must ultimately wait for the development of a suitable reinforcing matrix Recent trials with woven nylon tubes show promise It is hoped that this method for controlled neural regeneration may eventually find application in man as an aid in re establishing neural continuity reducing scar formation at suture lines and preventing neuroma formation in severed nerves

## CONCLUSIONS

1 Preliminary results of experiments using Millipore filter tubes to achieve bridging of long gaps in peripheral nerves are presented

2 Regeneration of Schwann tubule encased axons has occurred in an orderly fashion over a distance of 2 5 cm during a 3 to 4 wk interval following operation

3 The application of snugly fitted blind socks of Millipore to nerve stumps has prevented neuroma formation in short term experiments

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# SPONTANEOUS RETURN OF FUNCTION FOLLOWING SURGICAL RESECTION OF THE SEVENTH NERVE FOR PAROTID TUMORS*

HAYES MARTIN AND JAMES T HELSPER

The purpose of this preliminary report is to briefly present clinical evidence which shows that following surgical section and sacrifice of a considerable segment of the seventh cranial nerve (facial) there can be spontaneous recovery of function in a fair percentage of cases without resort to nerve grafting or other neurorrhaphy

The clinical basis for this report is an analysis of a consecutive series of 150 operative cases of malignant tumors of the parotid gland seen on the Head and Neck Service of Memorial Hospital during the period 1919 to 1953 inclusive Incidental to surgical removal of the tumors 40 patients (27 per cent) had a deliberate section of the 7th cranial nerve and excision of a segment of between 2 5 and 5 cm of the main trunk of the nerve and its dividing plexus (Fig 1)

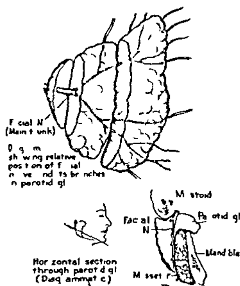



Fig 1 Diagrammatic representation of the relationship between the 7th cranial nerve (Facial) and the parotid gland

Of the 40 patients with such sacrifice of the nerve 28 were considered to be determinate The remaining 12 indeterminate patients (all with cancer of the parotid) had either rapid and massive local recurrences with gross invasion of the facial musculature or suffered generalized dissemination of cancer None of the latter 12 patients lived long enough to establish whether there would eventually have been any return of function

The noteworthy observation which we wish to place on record is that at least 7 patients (or 25 per cent) of the 28 determinate cases had a fair degree of return of function in the paralyzed facial musculature without nerve grafting or any other reparative operation

In Table I there is given a brief resume of the 7 cases of spontaneous recovery The patients ranging in age from 16 to 63 yr were all operated upon during a 5 year period (1949 to 1953 inclusive) As is the routine in our clinic the main trunk of the 7th nerve had been exposed as a preliminary step of the operation In all instances the decision to section the main trunk and to sacrifice the nerve was made only after it had been clearly

*From the Head and Neck Service Memorial Hospital New York N Y

*Table No 1 Pertinent data concerning the 7 patients in whom spontaneous return of function occurred following excision of a segment of the 7th cranial nerve (Facial) incident to surgery for parotid tumor*

| PATIENT | AGE | SEX | MINIMUM LENGTH OF RESECTED SEGMENT (CM) | DIAGNOSIS | FIRST RETURN OF FUNCTION (MO) |
|---|---|---|---|---|---|
| N B | 28 | F | 3 | Mucoepidermoid Carcinoma | 8 |
| L R | 16 | F | 4 | Adenocarcinoma | 6 |
| R F | 63 | M | 5 | Epidermoid Carcinoma | 6 |
| J A | 37 | M | 3 | Recurrent Mixed Tumor | 10 |
| M L | 26 | F | 2.5 | Mucoepidermoid Carcinoma | 8 |
| J K | 48 | M | 2 5 | Mucoepidermoid Carcinoma | 21 |
| J W | 61 | F | 2 5 | Fibrosarcoma | 13 |

demonstrated that the tumor was not resectable without such sacrifice Accordingly a segment of the main trunk of the nerve and of its adjacent plexus was removed with the tumor and a portion of the parotid gland

The lengths of the resected segments ranged from 2 5 to 5 cm No attempt was made in any of the 10 cases to resuture or to graft To relieve the more serious aspects of the orbicularis oculi paralysis a fusion of the eyelid was routinely made on the affected side at the completion of the main operation There was immediate and complete paralysis postoperatively of the corresponding facial musculature in all of the cases In the 7 cases of spontaneous recovery the patients themselves were the first to discover that some active motion had returned The time from operation to the first note of motion varied from 6 to 21 mo as shown in the last column of Table 1

The first evidence of motion in all patients had been the ability to pull back voluntarily the angle of the mouth Once initiated there was a progressive and steady improvement in the degree and extent of recovery Usually the muscles of the lower lip cheek eye and forehead in that order began to show voluntary motion

Once the voluntary motion returns to any part of the face it is as selective in that local area as in the normal opposite side The droop of the angle of the mouth so characteristic of 7th nerve paralysis completely disappears On forcible or exaggerated motion there usually remains some disparity between the 2 sides of the face but in repose and in ordinary facial motion the difference is so slight as to be unobjectionable We have reason to believe that such recovery of motion is favored and hastened if the patient actively and consistently (daily) practices in front of a mirror

One of the deterrents and handicaps in the detection and study of this phenomenon is the natural disbelief of the surgeon who having resected

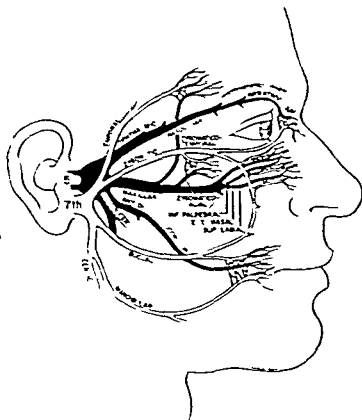



Fig 2 Diagrammatic representation of the nerve anastomoses between the 5th (Trigeminal) and the 7th (Facial) cranial nerves

a complete segment of the facial nerve cannot bring himself to accept the patient s claim of voluntary motion Another and even more difficult problem is presented when after a poorly conceived or unnecessarily traumatic operation is carried out a total 7th nerve palalysis ensues After a few months or longer function begins to return and the surgeon unreasonably concludes that he merely overstretched or crushed the nerve at the operation while what may have actually happened was that he severed the main trunk and a similar phenomenon as herein described has caused the return of function

In brief should there be functional disability of the 7th nerve (either partial or complete) after a parotid operation the actual physical status (section or lesser trauma) of the nerve cannot be known unless during the operation the main trunk and part of the plexus has been clearly exposed and identified and then either deliberately sacrificed or its continuity carefully preserved

From the purely theoretical standpoint there are several possible explanations for the return of function following complete section with loss of substance of the 7th cranial nerve

**Spontaneous Regrowth of Motor Fibers Across a Defect of Several Centimeters** We feel that the unlikely chance regrowth of fibers across a defect at least 2 5 cm in width from a single trunk over to many distal stumps and also in view of the fact that we have observed no mass motion in the affected muscles render such an explanation untenable

**Decussation and/or Anastomosis Across the Midline of the Face.** Since there is good selective motion of the involved muscles without motion of the contralateral muscles we believe this explanation also to be incorrect

**Establishment of New Motor Pathways Through the 5th Cranial Nerve**

(Trigeminal) According to anatomists the trigeminal nerve is divided into 3 divisions the ophthalmic and maxillary divisions thought to be purely sensory and the mandibular division which is both motor and sensory In addition there are described (usually in fine print) a number of anastomoses between the terminal branches of the 5th and 7th nerve whose precise functions are unknown These are shown diagramatically in Figure 2 Practically every major branch of the 7th nerve is supplied by an anastomosis with the 5th nerve What at first may seem a minor anatomical detail may actually have considerable significance Such terminal communications must obviously be closely associated with the motor end plates and to us it seems reasonable that following complete and permanent interruption of the motor pathway, impulses may find their way from the cortex through the 5th nerve to the respective muscles Although there may be some reluctance to accept this theory that new motor pathways may be established by way of the 5th nerve it seems to us to be the most reasonable explanation for the uncontestable fact that we have observed functional recovery in 7 cases despite segmental defects of several centimeters in the main trunk and plexus of the 7th cranial nerve

SUMMARY

1 Of 150 consecutive cases of malignant neoplasm of the parotid gland treated in the years 1949 to 1953 inclusive 40 patients had a segment of the 7th cranial nerve (facial) varying in length from 2 5 to 5 cm deliberately excised incident to their operation

2 Of these 40 patients 28 lived free of disease for over one yr

3 Of these 28 determinate patients 7 had a definite return of function after periods varying from 6 to 21 mo without nerve grafts or any type of neurorrhaphy

4 We believe this return of function to be due to a growth of motor fibers through the 5th cranial nerve to the anastomosis between the 5th and 7th nerves and thus to the involved muscles

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# THORACIC CORDOTOMY UNDER EPIDURAL BLOCK ANESTHESIA*

FREDERICK MURTAGH LEROY W KRUMPERMAN MARY RUTH WESTER

It has been well established that successful sustained relief of intractable pain by anterolateral cordotomy depends upon the establishment of adequate levels of complete analgesia at the time of the operation For this purpose local infiltration anesthesia supplemented with pentothal and nitrous oxide or Trilene has been advocated The patient is asleep during

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the initial phases of exposing the cord He is awakened at the time cuts are to be made into the cord Sensory testing is performed after the spinal thalamic tracts are sectioned and the areas of analgesia thereby produced are determined If necessary supplementary cuts are made until the desired levels of complete analgesia are obtained The patient is put to sleep again for the closure

Numerous operators have found this method unsatisfactory Thoracic laminectomy is a formidible procedure to perform under local infiltration anesthesia alone and often moderate to large amounts of supplementary anesthesia are needed to make the patient comfortable during the preliminary phases of the operation Under these circumstances it is often difficult to arouse the patient to the full levels of consciousness necessary to make subjective responses to sensory testing reliable In a state of partial dulling of the sensorium the difference between complete analgesia and a high grade hypalgesia may not be well appreciated by the patient An incorrect intrepretation of sensory loss may result In addition under local infiltration and with the patient awake manipulation of the sensory nerve roots is painful and distracting to the patient This pain may be so severe that the subjective response to sensory testing is further impaired The surgeon in his natural desire not to cause undue pain for the patient is restricted in his ability to manipulate the cord for proper cutting and he may technically defeat himself The advocates of the use of general anesthesia for cordotomy prefer the anatomic accuracy possible with the patient asleep

In order to provide total surgical analgesia at the operative site and still have the patient awake for sensory testing we have employed epidural block analgesia

Epidural analgesia is a segmental analgesia produced by the deposition of local anesthetic solution into the epidural space

The exact site of action of the epidural analgesics is not clear but 3 possibilities exist 1 diffusion occurs across the dura into the subarachnoid space and cerebrospinal fluid effecting a true spinal analgesia 2 the drugs act directly on dural covered nerve roots in the epidural space 3 the nerves are affected distal to the dural sheaths after they have left the intervertebral foramina producing a paravertebral block

## METHOD

Having received preoperative medication the patient is placed in the lateral decubitus position with the knees and neck flexed as far as possible The skin over the back is aseptically prepared A skin wheal is raised in the interspace between T 7 and T 8 or T 8 and T 9 A #16 gauge Touhy Huber needle is introduced through the skin wheal and directed cephalad The degree of cephalad direction will be determined by the anatomic considerations of the interspace chosen As the needle is introduced considerable resistance is commonly met at 3 points where ligamentous structures are encountered The first of these is at the supraspinatous ligament Definite but less marked resistance is encountered when the needle reaches the infraspinatous ligament The third point of principle resistance is encountered when the ligamentum flavum is reached After the needle has reached the ligamentum flavum the stylet is withdrawn and a drop of sterile procaine or saline is placed into the hub of the needle The needle is then

advanced carefully thru the ligamentum flavum until a definite snap is felt indicating penetration of the ligament and entry into the epidural space At this point the solution in the hub of the needle disappears into the shaft of the needle (the so-called drop sign of Gutierrez)

A #3 Fr polyvinyl catheter is now introduced thru the needle and directed cephalad, and advanced 3 to 4 cm After the catheter has been advanced sufficiently the needle is withdrawn over the catheter leaving it in the epidural space The catheter is now secured in place with adhesive tape in such a manner as not to be in the operative field

An infusion of 5 per cent dextrose in distilled water is started and an initial reading of blood pressure is taken At about the same time 2 cc of anesthetic solution is run into the epidural space This is a test dose and serves 2 purposes (1) if the catheter has inadvertently entered the subarachnoid space spinal analgesia will result (2) if the catheter is in the epidural space a narrow band of analgesia will be produced corresponding to the level of the tip of the catheter

After the test dose has shown the location of the catheter the initial epidural injection is made The volume of anesthetic solution used depends upon the number of segments to be blocked and the location of the tip of the catheter

In our experience if the catheter has been introduced as we have described an initial injection of 10 cc will produce analgesia extending from C-5 6 to T 9 10 Surgical analgesia will be complete in from 10 to 15 min

A variable fall in blood pressure can be anticipated—therefore it is advisable to administer a prophylactic vasopressor prior to the injection of the anesthetic solution into the epidural space The dose of vasopressor is determined by (1) the anticipated number of segments to be blocked (2) the patient s normal and preoperative blood pressures If the prophylactic vasopressor proves to be inadequate an intravenous drip of dilute neosynephrine ( 002 per cent sol ) will be most effective

The local anesthetic agents which may be used in this technique are (1) xylocaine (Lidocaine) 1 5 per cent with epinephrine 1 200 000 (2) cyclaine 1 5 per cent (Hexylcaine) and (3) 15 per cent pontocaine hydrochloride (tetracaine) We prefer xylocaine or cyclaine because the onset of analgesia is rapid (6 to 12 min as compared to pontocaine 15 to 30 min ) The duration of analgesia with xylocaine or cyclaine is from 45 to 75 min In some cases it may even be longer In the majority of cases the operative procedure will require from 1½ to 2½ hr , therefore we have injected a second dose of anesthetic solution just prior to the laminectomy By following this procedure we have always had complete analgesia for the duration of the operative procedure

All cases but one have been operated in the lateral recumbent position This has been found to be most convenient since it is the position in which the anesthesia was given and is most convenient for the anesthesiologist to attend and question the patient during the sensory testing If the procedure is a unilateral cordotomy the side to be cut is placed upward No inconvenience *has been encountered in doing bilateral cordotomy in this position*

Complete laminectomy is performed to provide adequate exposure of the cord For unilateral cordotomy the laminae of T 2 and 3 are removed and the dura is opened in an elliptical fashion to the side of the intended cord

section For bilateral cordotomy T 2 3 and 4 are removed The dura is opened in the midline and cut transversely on one side at the cephalic end and transversely on the other side at the caudal end of the exposure The dentate ligaments are cut on both sides from their attachment to the dura The ligament is then grasped with a long straight forcep and the cord rotated mesially to expose the anterior lateral segment The arachnoid is opened and an avascular area on the cord is chosen The cut is made with an unguarded bistoury The knife is inserted at the dentate ligament and directed toward the center of the cord It is advanced to a depth of 3 to 5 mm and then swept anteriorly to exit just in front of the anterior rootlets Sensory testing is then performed If supplementary cuts are necessary they are made and the patient retested until satisfactory levels of complete analgesia are obtained

Epidural segmental block has been employed as the method of surgical anesthesia for 9 thoracic cordotomies Five of these were bilateral procedures 2 were unilateral and in 1 case bilateral section of the spinothalamic tracts was carried out in 2 stages All have been for relief of intractable pain in the low back perineum or lower extremities secondary to either carcinoma or trauma

The operation was well tolerated by all patients including two who were poor operative risks because of associated cardiovascular renal disease

All of the patients were sufficiently awake and alert for reliable responses to sensory testing during the sectioning of the cord One patient on whom the bilateral procedure was performed in 2 stages was not able to give completely accurate responses to sensory testing at any time because of a severe emotional disturbance The operator Dr H T Wycis was satisfied however at the time of the operation that complete analgesia below T 7 was produced by the cuts into the cord This was substantiated by the repeated postoperative testing although the patient was not initially relieved of her original complaint of painful muscle spasm in the lower extremities

None of the patients exhibited undue discomfort because of the surgical manipulation Stimulation and maneuvering of the sensory roots caused no discomfort The cord could be easily mobilized in each case and accurate anatomical section of the spinothalamic tracts could be performed with assurance In spite of this in only 3 of the 14 individual anterolateral cord sections was a single cut sufficient to produce complete analgesia at a satisfactory level In the remaining 11 sections one or more supplementary cuts were necessary For the most part the supplementary cuts had to be made posteriorly toward the dentate ligament to produce analgesia in the last 3 or 4 sacral segments

In the majority of cases the area of segmental analgesia produced by the epidural block extended from C 7 or T 1 to T 8 or 9 In 1 case it ascended to C 4 producing respiratory embarrassment In 2 cases it descended to T 12 and L 2 respectively making it impossible to accurately determine the upper levels of analgesia produced

Seven patients obtained permanent relief of pain although in 1 of these cases there was a postoperative drop of 5 dermatomeres in the sensory level on each side It is worthy of note that 1 patient who had in addition to pelvic and generalized leg pain severe root pain in the fifth lumbar root

voluntarily informed us on the operating table that she could feel the sudden relief of the root pain at the moment the cord was cut She described the sensation as one of sudden exacerbation of the pain followed by immediate relief of all pain This patient maintained complete relief of all spontaneous pain, but on the second postoperative day developed hyperalgesia and dysesthesia to touch in the fifth lumbar dermatomere

A second patient voluntarily described sudden electric like pain in the corresponding leg at the moment the spinothalamic tract was sectioned on each side

## CONCLUSIONS

**Advantages** Epidural block analgesia is superior to general anesthesia in thoracic cordotomy because (1) it avoids the cumbersome technique of endotracheal anesthesia and (2) it produces complete surgical analgesia and yet allows the patient to be fully awake and responsive for sensory testing at the time cuts are made into the cord

Epidural block analgesia is superior to local infiltration anesthesia because (1) much smaller quantities of supplementary anesthetic agents are necessary (2) the operator is afforded complete freedom of movement and manipulation of the cord and nerve roots without causing distracting pain to the patient

**Disadvantages** Epidural analgesia requires the skill of a trained anesthesiologist *In the hands of an inexperienced operator the failure rate* may be high due to an inadvertent subarachnoid injection or inability to identify the epidural space

With this method analgesia is produced in the thoracic segments which may extend as low as T 12 or L 2 It may be impossible therefore if one is seeking a high level of analgesia by cordotomy to determine the upper levels at the operation However since this procedure is more often performed for pelvic and lower extremity pain this disadvantage may not be a serious deterrent to its use On the other hand if a high level is desired the anesthesiologist can make an effort to keep his lowest level of analgesia as high as possible

**Complications** Moderate to large falls in blood pressure may occur if too large a dose is used or if the proper prophylactic measures are not taken There may be systemic reactions to the local anesthetic agent if large doses are required to obtain the desired spread of analgesia These 2 disadvantages may be avoided by the proper placement of the catheter Respiratory distress may be present if the concentration of the drugs used is greater than described or if the upper level of analgesia extends upward to include C 3-4 5 resulting in paralysis of the diaphragm If a poor aseptic technique is used there could be contamination of the epidural space the meninges and the epidural space

**Contraindications** This technique is contraindicated if there is sepsis of the skin of the back at the site of the needle puncture and if there is known pathology in the epidural space or involved vertebrae It is unwise to introduce a catheter into the epidural space if the prothrombin time is below 35 per cent of normal

# Orthopedics

## THE RECORDING OF ROENTGEN IMAGES OF SMALL BONE SPECIMENS USING XERORADIOGRAPHY*

Eichiro Asai, Crawford J. Campbell, and John L. Roach

The recording of roentgen images of very small bones or fragments of bone from embryos or immature animals has been difficult.

Roach[1] in 1953 showed the superiority of xeroradiography in the study of wire screening of known size. In this method an electrostatic image produced on the surface of a semiconductor such as selenium is made visible by utilizing negatively charged powder granules.

Campbell, Roach and Grisolia showed how this resolving power of xero plates could be utilized to demonstrate bone detail in thin sections of normal and abnormal bone specimens. They confirmed the finding of Oliphant[2] and Hills and Stanford[3] that in a xeroradiograph there is a slight exaggeration of detail due to an increase of powder to one side of a charged area and a corresponding decrease of powder immediately adjacent to it.

The purpose of this investigation is to test the use of xeroradiography in the demonstration of bone detail in rat and human embryos.

### METHOD

Regular and two-fold enlargement xeroradiographs and conventional roentgenograms were taken of the following: 1. Rat embryos removed at weekly intervals from conception until maturity. 2. Human embryos of approximately 4 mo. of gestation. 3. Individual embryonic bones (human).

Enlargement radiographs were obtained by placing a small focus (0.3 mm.) tube 17" above the specimen and the plate exactly the same distance below. The original xeroradiographic images were recorded photographically using 35 mm. Type A Kodachrome film and were enlarged by the photographic method to the same size as the conventional roentgenograms.

### RESULTS

In the study of the rat embryos it is noted that at 10 days gestation there is no bone detail but soft tissue detail stands out more clearly in the xeroradiograph (Fig. 1 A, 1 B). Bone detail can be noted in the rib and skull in the xeroradiographs of the 2 wk. old embryo (Fig. 2 B) whereas a routine roentgenogram of the same specimen (Fig. 2 A) reveals only soft tissue. With further maturity the bone detail is always more pronounced in the xeroradiograph than in the roentgenogram (Fig. 3 A, 3 B).

Soft tissue detail is sometimes quite pronounced in the xeroradiograph as contrasted to the routine roentgenogram. This is well illustrated in the hemi

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Fig. 1 A Rat Embryo 10 days gestation Roentgenogram (50 MAS — 55 KV) enlarged 10 times by photographic technique

Fig. 1 B Xeroradiograph (50 MAS — 50 KV) enlarged 10 times by photographic technique



Fig. 2 A Rat Embryo 16 days gestation Roentgenogram (50 MAS — 55 KV) enlarged 7 times by photographic technique

Fig. 2 B Xeroradiograph (50 MAS 50 KV) enlarged 7 times by photographic technique



Fig. 3 A Rat Embryo new born Roentgenogram (50 MAS — 55 KV) enlarged 5 times by photographic technique

Fig. 3 B Xeroradiograph (50 MAS — 50 KV) enlarged 5 times by photographic technique

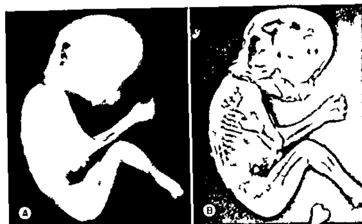


Fig 4 A Human Embryo approximately 4 mo gestation Roentgenogram (50 MAS — 53 KV) reduced in size by photographic method to 1/2 actual size

Fig 4 B Xeroradiograph (45 MAS — 60 KV) reduced in size by photographic method to 1/2 actual size

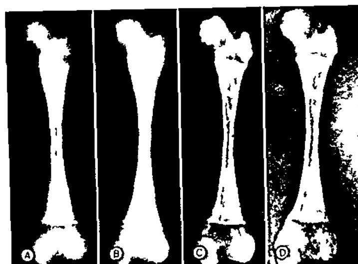


Fig 5 A Femur of human embryo approximately 4 mo gestation Roentgenogram (50 MAS — 53 KV) enlarged by photographic methods 2X

Fig 5 B Roentgenogram (50 MAS — 53 KV) enlarged 2X by radiographic enlargement technique

Fig 5 C Xeroradiograph (45 MAS — 60 KV) enlarged by photographic methods 2X

Fig 5 D Xeroradiograph (45 MAS — 60 KV) enlarged 2X by radiographic enlargement technique

section of the human embryo of 4 mo gestation in the demonstration of the ventricles of the brain, tongue larynx trachea and esophagus (Fig 4 A 4 B)

In the study of routine roentgenograms of embryonic bones better detail was obtained by enlarging the routine roentgenogram by photographic (Fig 5 A) rather than by radiographic enlargement technique (Fig 5 B) This may be accounted for on the basis of difficulty in getting good contrast In xeroradiography however the radiographic enlargement technique gave better detail (Fig 5 C 5 D)

## DISCUSSION

In this study of embryonic bone specimens the definition and contrast gradation in both bone and soft tissue in the xeroradiograph are more pronounced than in the conventional roentgenogram In the area of decreased deposit the fine detail may be diminished or obliterated This was called the pull in effect by Oliphant [2]

Artifacts in the form of small spots were often present They were frequent when the humidity in the atmosphere was high and on the older and more defective xeroplates Such artifacts although they obscured some detail were not easily confused with anatomic structure The humidity may affect the size of the granules thereby altering the clarity of the image

The image obtained in both bone and soft tissue detail in enlargement xeroradiography shows a contrast gradation superior to that obtained in conventional enlargement radiography

## CONCLUSIONS

Xeroradiography and enlargement xeroradiography are useful aids in the roentgenographic study of small bone specimens

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# THE HISTOGENESIS OF THE FUSION PROCESS AND THE USE OF TRANSVERSO FACET BONE BLOCKS IN SCOLIOSIS

J VERNON LUCK

It has been said that a successful research project ultimately poses more problems than it solves By this definition this research project involving scoliosis has already been successful

At the outset it was our hope to establish the manner in which the fusion segment grew in size following spinal fusions during childhood The study was soon expanded in an effort to understand the causes of stress pseudarthroses The incidence of pseudarthrosis following spinal fusions is distressingly high and it is natural that a histologic study of the fusion process would run head-on into the problem of the pseudarthrosis The pseudarthrosis aspect of the investigation has included a study of mechanical stresses that are brought to bear on the fusion segment particularly bending and torsion stresses This led us to use the transverso facet bone block a technique designed to create a compression arthrodesis particularly at the apex

of the primary curve where bending stresses are liable to develop to a high degree and thereby create a pseudarthrosis

**Histogenesis of the Fusion Process and Mechanism of Growth of the Fusion Segment** When events proceed through a normal sequence the steps by which a fusion is achieved are similar to the stages in the healing of a fracture Most of the callus that forms is bony in type although we have seen several instances of cartilaginous callus formed in a varying degree Fibrous bone forms abundantly and bridges the fusion segment Gradually the fibrous bone is replaced by mature lamellar bone completing the fusion process

There has been an interesting polemic through the years centered around whether or not the fused segment of the spine underwent growth Since there is no phenomenon of intrinsic growth in bone such as we see in cartilage it was reasoned that there could be no growth of the fusion block Obviously there are no enchondral lines to increase growth by enchondral ossification Theories to the contrary notwithstanding definite growth in the fusion block has been identified in numerous instances by Risser Hallock and others[1] The mechanism of this growth has not been clearly established

## METHOD

In the experimental phase of this study spinal fusions were carried out on white rats which were approximately one third grown A segment of Kirschner wire has been inserted to lay on the dorsal surface of the laminae extending above and below the fusion segment This was used for immobilization A second Kirschner wire was placed in the same location except that this second wire was cut the exact length of the fusion segment This gave an accurate index of the original length of the fusion

The study thus far has been a basis for two observations (1) The fusion block grows in size during growth (2) The mechanism of growth appears to be one of simple periosteal ossification This periosteal ossification appears in two forms (1) A layer of osteoblasts on the surfaces of the fusion block creates layer upon layer of periosteal new bone These layers are rather promptly assimilated and soon appear as lamellar bone (2) The second type of periosteal ossification is in the form of trabeculated periosteal fibrous bone This is particularly evident earlier in the fusion This fibrous bone forming on the surface of the fusion block and at each end is steadily replaced by mature lamellar bone In the past we have underestimated periosteal ossification as a mechanism of growth It is our present belief that this simple mechanism is the explanation of growth of the fusion block during childhood

**Stress Pseudarthroses** No attempt will be made here to present a detailed discussion of pseudarthrosis in spinal fusions Our study of this aspect of scoliosis is far from completed The study thus far relates to what we term the stress pseudarthrosis The most common type appears to be this stress type and it generally appears at the apex of the curve where substantial corrective forces have been exerted A damaging bending force can appear in the fusion block many months after the fusion procedure If the cast does not securely hold the correction achieved in the curve then bending forces frequently strong and inexorable try to recreate the original primary curve It is necessary to securely hold the curve in correction following the fusion

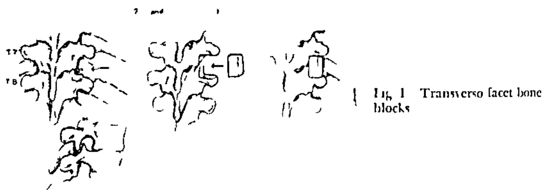



Fig. 1 Transverso facet bone blocks

until the fibrous bone created early by the fusion process is replaced by lamellar bone. Even with mature lamellar bone an osteolytic process can develop at the apex of the curve when the load of bending stress is extreme and the size of the fusion block is thin and narrow.

In general the greater the prefusion mobility the greater the incidence of pseudarthrosis but equally and probably even more important the greater the force required to correct the primary curve the higher the incidence. Older age groups have a higher incidence.

In general the longer the fusion area the higher the incidence of stress pseudarthrosis. It is therefore in good order to search for methods and *techniques that promise a reduction in the incidence of pseudarthrosis* and this led us to utilize transverso facet bone blocks.

**Transverso Facet Bone Blocks** It is now well established that compression forces are favorable and are capable of stimulating the fusion process. Likewise bending and torsion forces are capable of retarding and thwarting the fusion process. In studying the anatomy of the spine the close relationship between the facet joints and the transverse processes becomes evident. In the dorsal spine the transverse processes are readily visualized and a bone block *can be easily inserted between two transverse processes and against the denuded* facet joints. In the technique of preparing a bed and inserting the bone block the facet joint is clearly visualized as is also the base of the transverse process above and below the facet joint. Bone blocks are inserted

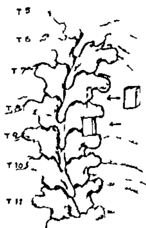



Fig. 2 Transverso facet bone blocks at the apex of a scoliotic curve on the concave side. Bone blocks are used in conjunction with a Hibbs fusion

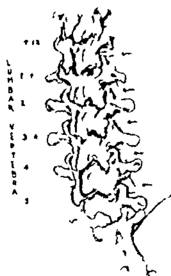


Fig 5 Transverso facet bone blocks in the lumbar region

on the concave side of the curve and in most instances have been limited to the apex of the primary curve At the base of the transverse processes a squaring is carried out and the margin of the facet joint excised With the squaring of the base of the transverse process above and below and the excision of the margin of the facet joint a bed is created to receive a rectangular block A bone block is then prepared and is taken from whatever source is desired Bone blocks have been prepared from a variety of sources which include the tibia the ilium, the spinous processes the ribs, and a segment from a fusion block created by a previous operation From 1 to 3 blocks are used and these are inserted at levels at and adjacent to the apex of the primary curve on the concave side The transverse processes are spread although not much spreading can be achieved as a rule The bone blocks are inserted by tapping them into place giving them a tight fit With increase in the bending stresses which so often happens postoperatively compression forces are increased at the most vulnerable site of the fusion segment which is the apex of the curve A second use has been made of the bone blocks by utilizing them in pseudarthrosis following conventional fusions After excision of the pseudarthrosis and the creation of multiple bone chips across the site of the pseudarthosis cleft a bone block has been inserted between the two transverse processes and against the facet joint directly at the site of the pseudarthrosis The results from this technique are most encouraging but further observation will be required and long range results studied before final conclusions can be drawn Transverso-facet bone blocks have been used in nine cases Three of these were pseudarthroses and six were in conjunction with Hibbs type spinal fusions

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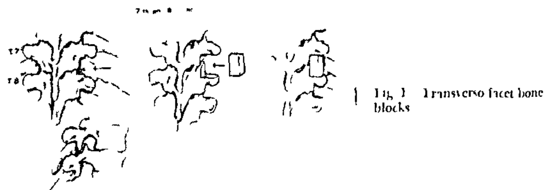


Fig. 1. Transverso facet bone blocks.

until the fibrous bone created early by the fusion process is replaced by lamellar bone. Even with mature lamellar bone, an osteolytic process can develop at the apex of the curve when the load of bending stress is extreme and the size of the fusion block is thin and narrow.

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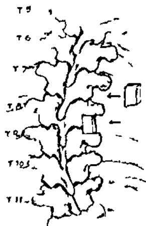


Fig. 2. Transverso facet bone blocks at the apex of a scoliotic curve on the concave side. Bone blocks are used in conjunction with a Hibbs fusion.

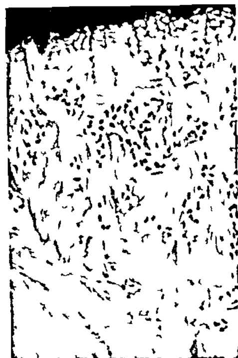


Fig. 1 Photomicrograph ×300 showing 70 day old rat after 49 days on a diet extremely low in calcium and vitamin D Epiphyseal cartilage appears across the top of the picture Mast cells appear in the endosteal connective tissue throughout cancellous bone Periosteum and muscle shown in the lower left side of the picture contain few or no mast cells Hematoxylin eosin and azure II stain

growth 4 to 6 wks later or at about 12 wks of age Mast cells appeared precipitously in the experimental animals at about 4 wks on the diet and rapidly increased in numbers until the bone was saturated with as many as 250 per high power field (×450)

Fracture healing (the formation of new bone tissue) occurred in these animals about as rapidly as in normal animals Callus formation was exuberant The fracture site seemed to hold the highest priority in the body for the building stones necessary for osteogenesis Calcification of the new bone and fibrocartilaginous callus was slightly delayed however and as in the metaphysis of the same bone there was a certain amount of osteoid and chondro osteoid There were no mast cells in the callus during the first 3 wks of healing when there were proliferating osteoblasts and while new bone formation was rapidly in progress After 3 wks of healing when osteogenesis was slowed down and marrow appeared in the callus mast cells began to appear first in small numbers outside the endosteum and then at greater numbers in place of the endosteum

Fifteen littermates reared on the experimental diets were treated with large doses of vitamin D (Calciferol 900 000 I U per cc daily for 7 days) The rachitic metaphysis was absorbed and mast cells were degranulated and disappeared into the bone marrow

Fifteen experimental animals were also treated with large doses of parathyroid extract (1 000 I U per day for a period of 7 days) Parathyroid hormone superimposed more extreme lesions of osteitis fibrosa upon the complex pathological picture of the bones caused by the calcium deficiency Osteitis fibrosa appeared to favor the formation of mast cells

## DISCUSSION

The function of the mast cell is not known The following substances have been claimed to form in the granules of mast cells histamine heparin

# MAST CELLS IN BONES*

MARSHALL R URIST AND FRANKLIN C MCLEAN

Striking formations of mast cells were encountered by chance in calcium deficient animals in the course of studies upon bone formation and repair [5] Mast cells are normally located in the bone marrow and rarely ever near bone tissue In rats fed a diet extremely low in calcium and vitamin D (Shohl s Diet E)[3] mast cells increased in numbers in the endosteum from 0 to 5 to 150 to 200 per high power field These observations were possible because of the authors routine use of hematoxylin eosin and azure II staining of undecalcified sections of all of their experimental material Hematoxylin and eosin the stains generally used for histological work on bone scarcely reveal the presence of mast cells The work presented in this paper began in 1937 and is submitted to this Forum because of the great interest in mast cells currently in all fields of biology and medicine

The bone disorder produced in rats fed Shohl s Diet E is a combination of moderate rickets (secondary to the vitamin D deficiency) osteoporosis secondary to the calcium deficiency and osteitis fibrosa resulting from stimulation of the parathyroid glands [1] (possibly by the low level of calcium in the serum) The rickets was evident by osteoid tissue around the bone trabeculae the osteoporosis by the low volume of bone and the attenuation of the trabeculae the osteitis fibrosa by the increased numbers of osteoclasts and the large amount of fibrous tissue between the trabeculae

The new location and the appearance of increased numbers of mast cells was not accompanied by an increase in the mast cell population in the mesentery of the same animals or the synovial membranes of the joints of the same bones in the same individual It is characteristic of the mast cell to *find it in clusters in various locations in the body as for example* (1) a membrane such as the pleura of mesentery (2) a perivascular connective tissue focus such as in skeletal muscle heart or visceral organs (3) a complex tissue such as bone marrow, (4) a pathological tissue such as a clinical or experimental tumor The endosteal location resembled the perivascular connective tissue location but it differed in that it had a distinct pattern of its own The mast cells were on the surface of within and even beneath the connective tissue cells lining the bone trabeculae There were however numerous mast cells surrounding small and large blood vessels adjacent to bone tissue In young growing animals osteoblasts are normally found in this location

## METHOD

Mast cells accumulated in the bones in calcium deficient rats when growth was arrested between 3 and 4 wks after the animals had been fed the experimental diet Rats reared on control diets reached the plateau of their rate of

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*From the Division of Orthopedics Department of Surgery University of California Los Angeles California and the Department of Physiology University of Chicago Chicago Ill Aided by grants from the Josiah Macy Jr Foundation Ayerst Research Council Lee Mytinger Foundation of the University of California Los Angeles National Institutes of Health and Public Health Service and the Wallace C and Clara A Abbott Memorial Fund of the University of Chicago

seventh decades. Hence, despite the absence of quantitative experimental evidence, it seems apparent that the difference in velocity of healing is greater between infant and adult in fracture healing than in soft tissue healing. It also seems apparent that fracture healing velocity may reach a plateau in late adolescence and early adult life with little if any subsequent diminution in velocity.

If these opinions are true we must look elsewhere than to the chronologic age of bone alone as an explanation. The epiphyseal cartilage has a life history which parallels the increased velocity of fracture healing in the growing animal. It is at least logical, therefore, to suspect that the governing factor regulating fracture healing velocity may either reside in the epiphyseal cartilage or accompany its presence. The occurrence of pathological fractures in the long bones of weanling rats as differentiated from adult animals when exposed to massive doses of Vitamin A is well described and discussed by Wolbach.[3] This fact is additional evidence that the epiphyseal cartilage is not only an anatomic unit contributing to bone growth in length but may be a physiologic unit upon which other aspects of bone physiology depend.

## METHOD

To investigate this, fractured femora in growing and late adolescent guinea pigs were examined utilizing Vitamin A in excess as a tool to unbalance normal epiphyseal physiology.[3, 4]

Vitamin A in oil in a 750 000 international unit per cc. concentration was administered 3 times weekly in a dosage to be equivalent in total to 500 international units/gm./day. The drug was not begun until 1 wk. after fracture and the oil preparation and intermittent dosage were utilized to limit



Fig. 1. Gross appearance of section through untreated infant femoral shaft fracture. The callus nidus is apparent even at this minimal magnification and is cartilaginous.

hyaluronic acid or some other acid mucopolysaccharide precursor of ground substances acid phosphatase alkaline phosphatase phospholipid, lipids, lipase peroxide, cytochrome c oxidase unidentified proteins free iron Of these substances alkaline phosphatase and ground substance precursors are 2 that could be stored in mast cells when bone formation ceases in calcium deficient animals

The mast cell requires further study in animals and humans with various disorders of bone such as rickets osteomalacia osteoporosis and osteitis fibrosis Its role could be to store alkaline phosphatase but it is actually as much of a riddle in bone as in all of the other connective tissues of the body [4]

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# THE INFLUENCE OF VITAMIN A INDUCED EPIPHYSEAL CARTILAGE ABNORMALITIES ON FRACTURE HEALING IN THE GUINEA PIG*

EDWIN G BOVILL JR

The velocity of the healing process in skin and in fibrous connective tissue can be expressed quantitatively and undergoes a steady decline with age [1, 2] The velocity of healing in a soft tissue wound in the young animal is only a fraction faster than in the adult The young adult will heal a soft tissue wound measurably faster than the older adult I agree with Harvey[2] that healing of a soft tissue wound appears to involve reactivation of a normal growth process

A difference seems apparent on comparing fractures with soft tissue wounds from the point of view of velocity of healing Quantitive methods of measurement of fracture healing are not available Clinical information in the human substantiates the opinion that long bone fractures in the first decade of life heal at least twice as fast as comparable fractures in the third and fourth decades and possibly three times as fast Furthermore very little if any comparable difference in velocity of healing is present when comparing fractures in the third and fourth decades with those in the fifth sixth or

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guinea pigs (225 gm. ± 10) were utilized. Digital fracture of the right femoral shaft was carried out on all animals and they were then separated by random selection into 4 groups of 10 each. Ten of each age group received no treatment. The other 10 of each age group received Vitamin A in oil as outlined above. At 5 wks. all animals were sacrificed. Microscopic sections were prepared in the longitudinal axis in either sagittal or coronal plane, whichever best permitted the section to pass through the center of the fracture and through the distal femoral epiphysis.

Seven of the 40 animals were lost to the end result either through death early in the experiment or by presenting hip dislocation, epiphyseal separation or no fracture at the time of sacrifice and examination. The one distinction sought was the presence of either cartilage or fibrous connective tissue as the fracture callus nidus.

*Fracture Callus Nidus, Infants—*

| Treatment | Cartilage | Fibrous Con. Tis. | |
|---|---|---|---|
| Control | 7 | 0 | 7 |
| Vitamin A in Excess | 4 | 6 | 10 |
| | 11 | 6 | 17 |

*Fracture Callus Nidus, Adolescents—*

| Treatment | Cartilage | Fibrous Con. Tis. | |
|---|---|---|---|
| Control | 7 | 0 | 7 |
| Vitamin A in Excess | 7 | 1 | 8 |
| | 14 | 1 | 15 |

The treated 3 wk. old animals and the untreated and treated adolescent animals did not change weight appreciably over the period studied. The untreated 3 wk. old animals gained to an average weight of 100 gm. ± 50.

A similar experiment using tibial fractures was characterized by the absence of a cartilaginous bar in the callus in any of the animals. This appeared to be the result of close apposition of the fragments in the tibial fractures as compared with the rather wide separation typical of the femoral fractures. The tibial fractures in general demonstrated early trabecular bony bridging of the fracture at 5 wks. No definitive factor was found in the tibial fractures to permit comparison of the treated and untreated animals in a quantitative fashion.

SUMMARY

Previous experimental work with excessive dosage of Vitamin A has demonstrated that some of its action on bone may be the result of Vitamin A induced epiphyseal cartilage changes rather than direct.[3] The results in the group of femoral shaft fractures reported here agree with this concept.

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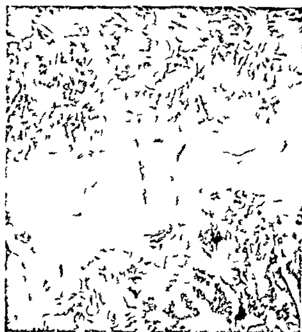


Fig 2 Section through center of callus nidus in treated late adolescent Cartilage

the amount of handling to a minimum That the vitamin was active was evidenced by the epiphyseal plate response which was always present although not of uniform degree The lack of uniform response may represent uneven absorption from the depots of injection

The lack of a definable end point to fracture healing prevented measurement of velocity of healing At the 5 wk post fracture period all untreated animals exhibited a bar of cartilage between the fragments in the center of the callus This was not uniformly true with the young treated animals as the central portion of the callus was frequently fibrous connective tissue Accordingly the following experimental design was used

Twenty late adolescent guinea pigs (550 gm $\pm$ 50) and 20 three week old

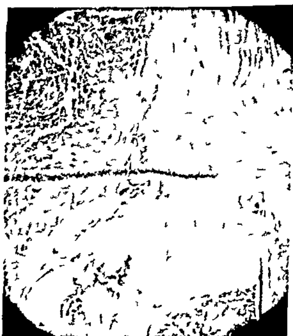


Fig 3 Section through center of callus nidus in treated infant Fibrous connective tissue

RESULTS

Observation of the injected joints of the sensitized animals during the first few hours revealed progressive swelling of both joints causing the animals to limp badly Measurements of the amount of swelling were not carried out There was less swelling of the joints of the non sensitized animals these animals likewise limped Freund[4] had previously shown that the paraffin oil in itself is a tissue irritant

Gross examination of the joints in the sensitized group after sacrifice revealed marked engorgement of the blood vessels about the knees more marked in those containing the adjuvants There was gross swelling of all the joints but most in the adjuvant containing joints There was injection of the blood vessels of the controls also which was the most marked feature of the reaction of these joints

Examination of the joint cavities of the sensitized group revealed a great increase in the amount of synovial fluid In contrast the joint cavities of the control animals were dry free of any synovial fluid with the adjuvant clinging to the synovium and difficult to wash or pull free At 24 hr the synovial fluid in the sensitized animals was bloody This did not occur in those sacrificed at later dates though the overall amount of synovial fluid was increased At 24 hr in the sensitized group the synovium was markedly thickened beefy red in color and encroached on the articular cartilage and the patella (Fig 1) The adjuvant containing joints showed the more marked reaction

The reaction noted in the knee joints of the sensitized animals decreased from a marked reaction at 3 days and gradually decreased through the 28th day (Figs 2 & 3) In the non sensitized control animals there was never any synovial reaction or increased fluid except where the dry adjuvant was adherent to the synovium One control animal had a grossly infected joint but the synovium even in this joint did not compare with those previously sensitized (Fig 4)

Histological sections of the synovium of the animals in which an anaphylactic arthritis was produced showed a greatly thickened synovial lining with villous formation and a marked inflammatory reaction as evidenced by the tremendous number of inflammatory cells (Fig 5)



Fig 1 Severe synovial reactions in the knees of a sensitized rabbit 24 hr after injection of 0.5 cc of Freunds adjuvants in the knee on the left and 0.5 cc egg albumin *into* the knee on the right

# THE PRODUCTION OF ANAPHYLACTIC ARTHRITIS WITH THE USE OF FREUND'S ADJUVANTS*

RICHARD T ODELL AND J ALBERT KEY†

Local anaphylaxis or an Arthus phenomenon was demonstrated by Arthus in 1903[1] This phenomenon or local anaphylaxis is the inflammatory reaction occurring when an animal is immunized against a protein and is re-injected with the same antigen i e the reaction which occurs when antibody and antigen meet in the tissues Friedberger[2] is generally credited as the first to produce local anaphylaxis in the joints Many investigators have been interested in producing as well as preventing or controlling anaphylactic arthritis in experimental animals

Freund[3] and his coworkers found when they were elaborating on some older experiments that killed tubercle bacilli in paraffin oil produced complement fixation antibodies in high titer for at least 18 mo after injection Diverse antigens such as proteins bacteria viruses rickettsiae and simple chemical compounds combined with paraffin oil alone enhanced and prolonged antibody formation but had little effect on sensitization The addition of killed mycobacterium strikingly promoted sensitization with questionable increase in antibody production[4] Thus Freund produced a water in paraffin oil emulsion to which could be added a variety of antigens and produce antibody formation and sensitization The water in oil emulsion with killed mycobacterium frequently bears the name of Freund's adjuvants

In this experiment we used the adjuvant containing killed mycobacterium butyricum and added a protein antigen egg albumin Furthermore quite by accident the adjuvants were injected into the knee joint of a previously sensitized rabbit and the animal received such a severe reaction that we decided to continue the use of the adjuvant in the production of anaphylactic arthritis

## METHOD

A group of young adult white rabbits of the same sex were selected and each received intramuscular injections of 2 0 cc of Freund's adjuvants containing a 2 4 per cent solution of egg albumin One cc of the solution was injected into each of 2 sites deep in the muscles At the end of 3 wks skin tests with 0 1 cc of the antigen injected subcutaneously gave each of the animals a typical Arthus reaction The animals were then sensitized enough to have injections made into the knee joints One half (0 5 cc) of the egg albumin antigen was injected into the right knee and 0 5 cc of the adjuvants injected into the left knee A control group of animals which had not been sensitized received 0 5 cc of the adjuvants into the knee joints

The animals were observed for several hours on the day of injection of the joints and sacrificed with an overdose of ether at 24 hrs 3 5 7 10 28 and 90 days respectively Studies were made of the gross specimens which were then photographed and histologic sections made of the synovium fat pads and any damaged articular cartilage

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†Deceased



Fig 5 Typical inflammatory reaction and villous formation of the synovium following sensitization and injection of Freund's adjuvants into the knee joint of a rabbit

## DISCUSSION

The production of anaphylactic arthritis or local anaphylaxis in laboratory animals has long been of interest to immunologists The rediscovery by Freund that antigens incorporated in a water-in-oil emulsion and paraffin oil potentiated and prolonged antibody formation for long periods provided an excellent method for the local production of anaphylaxis Local anaphylaxis or the Arthus phenomenon has been produced in the integument the lungs livers and joints of animals We had not seen well illustrated local or anaphylactic arthritis and by accident discovered the use of the Freund adjuvant technique as a means of producing this type of arthritis We have succeeded in producing a severe anaphylactic arthritis in sensitized animals with Freund's adjuvants containing mycobacterium butyricum and egg albumin as an antigen The adjuvants without the antigen produced a greater and more severe reaction than the antigen proper Furthermore a chronic arthritis was produced when the sensitized animal's knee was injected with the adjuvants and the animal allowed to live for 3 mo or longer before it was sacrificed The histological picture in the acute anaphylactic stage was one of acute inflammatory synovitis

## CONCLUSIONS

1 A different method of producing anaphylactic arthritis has been shown

2 The use of Freund's adjuvants in the knee joints of previously sensitized animals consistently gave a severe local or anaphylactic arthritis

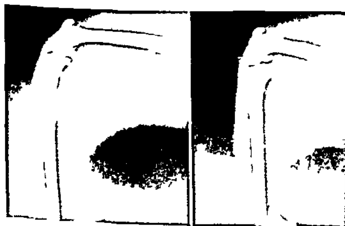


Fig 6 This roentgenogram shows erosion of the articular cartilage of the bones of the right knee 90 days after the injection of adjuvants into a sensitized rabbit The control left knee received no injection and shows no arthritis




Fig 2 The reaction at 3 days On the right there is virtually no reaction to the egg albumin antigen The reaction to the adjuvants in the knee on the left is quite severe




Fig 3 The 7 day reaction in sensitized animals is subsiding but is still considerable

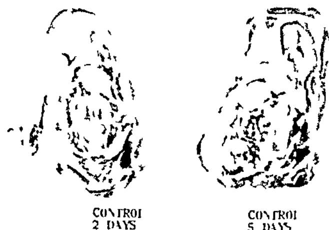



Fig 4 Control knees showing irritative power of the paraffin oil The reaction was greatest at 5 days

Several sensitized animals were kept for 3 mo after injection of their knee joints to see if a chronic arthritis could also be produced Figure 6 shows the arthritis produced in the right knee with the normal left knee as a control In the injected knee erosion of the articular cartilage of all the bones is seen and bony overgrowth is beginning to occur

extract of skin from 7 rabbits and received a graft from a donor not contributing to the pool. In the second experiments the donor rabbit provided the skin for both the extract and transplant. In the preparation of the pooled extract, as much full thickness skin as could be obtained was removed from the rabbits after clipping the hair and chemical depilation. The skin was minced after removal of all subcutaneous tissue and blood vessels and ground in a stainless steel ball mill at −70° C until the skin was converted to a fine powder. It was then extracted with buffered saline in a Waring blendor at 4 C. The extract was sterilized by Seitz filtration. It contained 8 mg. of protein/ml.

Each rabbit received autogenous and homogenous transplants. A 2 × 8 cm full thickness area of skin was removed from both sides of the posterior thorax. The panniculus carnosus was left as the host bed. Each piece of skin was cut into eight 1 cm squares. Autografts were placed on the right side and homografts on the left. Exchange of homografts were effected by operating on pairs of rabbits. The graft areas were covered with vaseline gauze, a gauze bolster tied over this and a light plaster bandage around the body. Either half or a whole graft was removed every 4 days for histological study.

Immunization consisted of 3 weekly subcutaneous injections of skin extract containing 25 mg. of protein per injection with Ramon's adjuvant. Serum specimens were taken from recipient animals prior to the grafting operation and at weekly intervals thereafter. One group of rabbits was grafted at the start of immunization and another group was grafted 5 wks after the start of immunization. Three rabbits were used as controls without immunization.

The individual extracts were prepared from large sheets of skin removed from the flanks of donor rabbits. The wounds were closed so that these rabbits could be used later as donors for skin grafts. In this 1 to 1 series all rabbits were grafted 5 wks. after the start of immunization since this was the time of occurrence of peak titer in the bone immunization studies.

RESULTS

In the control rabbits the usual findings in autogenous and homogenous full thickness grafts were observed. The homografts turned dark brown to black and showed complete necrosis from mid dermis outward by 12 days. About 15 per cent of the full thickness autografts showed failure of take of the epidermal elements.

In 3 rabbits grafted at the start of injections of extract there was no difference in the fate of the homografts in treated and control animals.

Two rabbits were grafted 5 wks. after the start of immunization with pooled extract and 9 rabbits with individual donor extracts. Since the gross and histologic results were similar, they will be described together. Only those transplants which adhered to the host bed at the time of first biopsy are considered. At 4 days the gross and histologic appearance of the autograft and homograft were similar. At 8 days a light tan to purple color appeared in a few of the homografts whereas most of the autografts were pink. They both recovered color after blanching on pressure. Microscopically the usual autograft epidermal hyperplasia was evident while the homograft epidermal elements showed pyknosis of cells with infiltration of

3 These same adjuvants can be used to produce a chronic arthritis in previously sensitized animals

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# THE FATE OF HOMOGENOUS SKIN TRANSPLANTS IN RABBITS IMMUNIZED WITH SKIN EXTRACTS*

M BONFIGLIO W S JETER AND W OSTRANDER

Attempts to obtain permanent survival of skin homografts in normal animals have met with uniform failure It is well known that skin homografts incite an inflammatory reaction which leads to necrosis and slough of the graft at a predictable time Efforts to modify the host reaction to skin homografts by injection of donor skin extracts have been reported recently Allen and associates[1] reported prolongation of survival of skin homografts in rabbits by desensitization with saline extracts in which phenol was added as a preservative Billingham and Medawar[2] however were unable to effect the survival time of skin homografts with phenol free donor skin extracts On the other hand Hardin and Werder[3] produced permanent survival in the majority of homologous skin transplants in heterologous CFW mice by subcutaneous injection of homologous skin extracts They considered the results to be due to a process of immunoparalysis rather than desensitization Previous studies on bone homografts by us[4] have shown that pre immunization of recipient rabbits with extracts of donor rabbit bone appreciably reduce the inflammatory reaction to a subsequent frozen homogenous bone graft

With these results in mind experiments were undertaken to see what effect injection of skin extracts would have on the fate of homogenous skin transplants in the rabbit

Using skin as donor material the techniques previously developed for bone extracts were employed[4]

METHOD

Large young male adult rabbits were used Two series of experiments were performed In the first series the rabbits were immunized with an

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*From the Depts of Orthopedic Surgery and Bacteriology College of Medicine State University of Iowa Supported by Public Health Service Research Grant No A 732 (C4) Surg National Institute of Arthritis and Metabolic Diseases National Institutes of Health United States Public Health Service

# THE EFFECT OF INTRAMEDULLARY NAILING ON THE GROWTH RATE OF IMMATURE CANINE FEMURS*

Frederick L. Behling and Joseph M. Janes

With the advent of the use of intramedullary fixation in the treatment of fractures many theoretical and practical problems have been introduced One of these concerns the effect of metallic intramedullary fixation on the epiphyseal growth in an immature long bone Blount[1] referred to the stimulation of the nail alone as being probably responsible for 1 to 2 cm of overgrowth of the immature femur in cases of fracture treated by intramedullary fixation reported to him Bisgard[2] in 1936 reported that fractures of the tibia in immature goats were found to produce more overgrowth in healing if internal fixation was used than if it was not used He based this conclusion on results of increased overgrowth found in 2 cases in which a Lane plate was used for fixation

By means of animal experiments we sought to evaluate the effect of metallic intramedullary fixation on the growth rate of an immature long bone hoping to get a clearer conception of the role of the nail as an alleged irritant to the growing epiphysis We selected the femur of the immature dog as a suitable bone In each animal we operated on one femur leaving the other unoperated as a control In one group of animals the femur was fractured and the fracture fixed by intramedullary fixation and in the other group the nail was placed the length of the intramedullary canal without producing a fracture

## MATERIAL

In this experiment 21 young dogs taken at random were used In most the ages were not exactly known but were estimated to be between 4 and 6 mo at the beginning of the experiment The estimates of age were made by the veterinary staff of the Mayo Foundation

The surgical procedures were performed with the animals under general anesthesia aseptic technique being used throughout The intramedullary fixation was accomplished by means of stainless steel Steinmann pins 3/32" in diameter shaped roughly to a point at either end and inserted by means of a hand drill

## METHOD

In all animals one femur, alternately left and right was operated upon the other serving as a control In one animal nonunion developed owing to a technical error and the intramedullary nail was extruded at the knee In another animal a massive infection of the operated leg developed These two animals are not included in the analysis of the experiment

Group I consisted of 10 animals in which an intramedullary nail was inserted from proximal to distal without fracture of the femur The superior aspect of the femoral neck was approached through a 1" incision and a 1/16" drill hole was made in the superior cortex of the femoral neck The Steinmann pin was drilled down the medullary canal until it could be

* From The Mayo Clinic and Mayo Foundation Rochester Minnesota The Mayo Foundation is a part of the Graduate School of the University of Minnesota

inflammatory cells about these elements By 12 days the homografts had become darker tan but not quite as leathery as the control homografts A few grafts had proliferation of epidermis and hair follicles as well as infiltration of inflammatory cells However most of the homografts at this time showed sufficient necrosis and inflammatory exudate at the junction of the deepest epidermal elements and the dermis to indicate that this portion of the graft would slough About two thirds of the dermis appeared viable At 16 days the superficial necrosis of the homograft was certain In a few homografts hyperplasia of the matrix cells of the hair follicles persisted as long as 16 days From this time on there was gradual marginal invasion of the junction between necrotic and viable dermis by host epidermis until the eschar was completely loose at an average of 24 to 28 days

In those instances where partial necrosis and slough of autografts occurred autograft repair appeared identical to the repair of the homografts up to 28 days The repair epidermis then developed projections into the dermis which appear like early hair follicles and sebaceous glands by 36 to 40 days Indeed homografts on 3 of the rabbits did grow fine hair Apparently this arose from regenerating host epidermis which covered graft dermis as recently shown by Breedis [5] The dermis of the homografts shrank gradually to about half the size of the autografts As late as 134 days there was persistence of homograft dermal collagen in the immunized rabbit

## CONCLUSIONS

1 None of the epidermal elements of full thickness homograft survived transplantation beyond 16 days in rabbits injected with pooled skin extract or donor skin extract with adjuvants

2 The homograft dermis in immunized rabbits persisted as long as 134 days

3 Regenerating host epidermis which covered the homograft dermis developed hair and sebaceous glands

4 Repair of partially necrotic full thickness autografts was similar to that of homografts

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Fig 3 Six weeks postoperative Group 1 femur showing growth about nail

were taken in this group also and follow up roentgenograms were taken in both groups to determine the progression of healing (Figs 3 and 4)

Following the surgical procedures the animals were returned to the kennels where they were given no special care other than 1 or 2 daily exercise periods Considerable postoperative reaction was noted especially in the fractured femur group It was noted that the operated legs were not used as weight bearing members in either group for about 1 wk after which all animals began to touch down on the operated leg The gait was normal



Fig 4 Two mo postoperative Group 2 femur showing healing and growth about nail



Fig 1 Immediate postoperative Group 1 femur showing nail in place

palpated just emerging from the bone distally It was then withdrawn to leave the pin just under the cortex distally The pin was then cut off proximally as nearly flush with the cortex as possible and the incision was closed (Fig 1) Postoperative roentgenograms were taken to determine the position of the pin especially with respect to the distal femoral epiphysis

In Group 2 consisting of 9 animals the femur was approached laterally and fractured in its mid third by means of a Gigli saw The intramedullary nail was then inserted in the usual retrograde manner After reduction of the fracture the nail was inserted to emerge at the distal end of the femur and then withdrawn as in Group 1 (Fig 2) Postoperative roentgenograms



Fig 2 Immediate postoperative Group 2 femur showing fracture and nail in place

*Table 2 Intramedullary Nailing Group Averages*

| AVERAGE | FEMUR FRACTURED | INTACT |
|---|---|---|
| Length of control femurs cm | 14.5 | 16.3 |
| Shortening after operation cm | 0 47 | 0.35 |
| Per cent of control | 3.2 | 2 1 |

is crossed by the nail In only 4 femurs as shown by the asterisks were the nails not across the epiphyseal line and this may not be a large enough group to serve as a basis for judgment

The important and statistically valid finding presented here is the fact that shortening occurred in all femurs with intramedullary fixation with or without fracture Table 2 shows a comparison of the 2 groups with group averages for length of the control femurs shortening of the operated leg and shortening as a percentage of the length of the control femur The shortening is greater in the fractured femurs as a group than in the intact femurs and the degree of shortening expressed as percentage line 3 also is greater

COMMENT

Our purpose to assess the influence on growth of metallic intramedullary fixation in a growing bone is achieved in the finding of shortening in all but one of 19 immature canine femurs into which we introduced stainless steel Steinmann pins with and without fracture Why the stimulation usually seen in humans and reported by Bisgard in animals following fractures of immature long bones was not seen by us is not known Not only was there no growth stimulation in fractures but rather the series of fractured femurs was shorter as a group than the series in which the intramedullary nail was introduced without fracture The fact that shortening was found in 18 of the 19 animals studied including 10 femurs without fracture disputes the role of the intramedullary fixation device as an irritative factor on the epiphysis

CONCLUSIONS

On the basis of these experiments we conclude

1 The presence of a metallic intramedullary nail in an immature canine femur does not produce an increase in length by virtue of its presence alone

2 A slight shortening follows intramedullary nailing of immature canine femurs slightly greater in bones which are fractured than in those without fracture

Further investigation is needed to correlate these findings with comparable experiences in clinical cases

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*Table 1 Shortening of Femur After Intramedullary Nailing*

| NUMBER | TIME MONTHS | SHORTENING OF FEMUR AFTER OPERATION Cm | PER CENT OF CONTROL |
|---|---|---|---|
| | | FEMUR NOT FRACTURED | |
| 358 | 6 | 0.7 | 4.2 |
| 357 | 5 | 0.5 | 3.5 |
| 501 | 5 | 0.5 | 3.4 |
| 353 | 5 | 0.5 | 2.9 |
| 354 | 5 | 0.4 | 2.4 |
| 355 | 5 | 0.4 | 1.7 |
| 600 | 2 | 0.2 | 1.2 |
| 352 | 5 | 0.2 | 1.1* |
| 498 | 5 | 0.1 | 0.6 |
| 603 | 5 | 0.0 | — |
| | | FEMUR FRACTURED | |
| 500 | 6 | 0.7 | 5.5 |
| 441 | 4 | 0.7 | 5.3 |
| 445 | 5 | 0.6 | 4.1* |
| 442 | 5 | 0.5 | 3.5 |
| 602 | 6 | 0.4 | 3.2 |
| 494 | 5 | 0.4 | 2.9 |
| 601 | 5 | 0.4 | 2.5* |
| 599 | 2 | 0.3 | 1.8* |
| 495 | 5 | 0.2 | 1.2 |

*Nail not across epiphyseal line

and no evidence of protection was evident in Group 1 at 3 wks and in Group 2 by 6 wks

The animals were killed from 2 to 6 mo after operation All animals were killed at more than 10 mo and less than 1 yr of age at a time when epiphyseal growth had nearly been completed

After death the hind legs were dissected free of soft tissue sections were taken of the epiphyses and the bones were boiled to rid them of all soft tissue Accurate caliper measurements were then made of the tibias and femurs in each animal These measurements were repeated by a disinterested observer to minimize technical error These measurements form the basis for analysis in this study The tibias were not found to differ by a significant amount and no trend was noted in the differences that did exist Study of the microscopic sections of the epiphyses showed no difference between the 2 groups The results of femoral measurement showed a statistically significant difference between the 2 groups and are shown in Table 1

Shortening occurred in all but one operated femur in the entire series and in that one the operated and the control bones were of the same length The amount of shortening compared as percentage to the length of the control femur is shown in the right hand column This column denotes the degree of shortening produced by the nail and the fracture and nail respectively

The degree of shortening is not directly related to the time the nail was in place and seems to be unaffected by whether or not the epiphyseal line



Fig 1

union 1 dog died from a severe infection 2 dogs in whom the ligamentum teres was preserved had contrasting stripping procedures on the capsule 1 dog on whom the minor stripping was done healed in 34 days the other dog on which a major stripping was done developed aseptic necrosis (Fig 1)

Forty three animals had either omental or muscle (vastus gluteus rectus) grafts applied to the head or neck fragments immediately following fixation A 3/16 drill hole was made in the distal fragments in an oblique direction as close to the fracture site as possible in some animals and subcapitally in the proximal fragments in others the various grafts were introduced into the drill tract and maintained by 1 or 2 silk sutures

Six animals had vastus lateralis muscle transplantation in 1 we used the gluteus minimus 5 had omental transplants to the middle of the neck area and 5 others had vastus lateralis grafts affixed to the sub capital area 3 had rectus abdominis grafts which included a branch of the inferior epi gastric artery Ten other animals had omental grafts placed just behind the head Thirteen animals on whom various procedures were done died from a variety of causes

## RESULTS

Our most difficult complication was a high infection rate This has been lowered considerably by the use of pre and postoperative cathomycin Mange bothered a large number of the animals and contributed to the mor tality rate Two hips dislocated one of which went on to apparent union while the other developed aseptic necrosis

## SUMMARY AND CONCLUSIONS

When the circulation of the neck of the femur is extensively damaged accurate reposition and fixation will not prevent non union and aseptic necrosis Omental grafts though they bring in circulation carry a high infection potential Vastus lateralis gluteus and other muscle grafts placed in the distal fragment near the fracture site are not as successful as sub capital grafts The most gratifying results have come from the rectus ab- dominis grafts placed in the head fragment It is the latter variety that we intend to use in our next experimental series

Circulation can be introduced into the heads of fractured femurs aseptic

# THE DEVELOPMENT OF CIRCULATION IN THE FEMURS OF DOGS AFTER FRACTURE OF THE FEMORAL NECKS*

CHARLES J. FRANKEL, DAVID V. STRIDER AND W. CLARKE POLL

The unsolved fracture, so aptly described by Kellog Speed in 1941, still remains the nemesis of surgeons everywhere. Non-union and aseptic necrosis have been attributed by Tovee and Gendron[1] to inaccurate reduction, poor nailing or too early weight bearing. Stuck and Hinchey[2] concluded that vascular damage was primarily responsible for aseptic necrosis; they devised muscle transplanting procedures which were successful in a few animals but proved to be inadequate for humans.

If Tovee's conclusions and inferences are correct, further research into the problems of introducing circulation into the heads and necks of fractured femurs would be unnecessary. The anatomy of the femoral head and neck of the dog is somewhat different from the human. In the latter there is always a well developed neck which has proven to be an obstinate barrier for circulation to cross in a proximal direction. In dogs and in other animals the trochanteric circulation provides a speedy process of creeping substitution since there is little or no neck intervening. The explanation for Tovee's excellent results, which he attributed to firm and accurate fixation, is probably on the basis of unimpaired circulation. It is completely misleading to draw any inference of badly damaged circulation from a procedure in which the capsule is not adequately cut, the head removed and then replaced and fixed securely. It is necessary to strip the capsule extensively in order to stimulate some of the Pauwels Type Three displaced fractures in humans.

Our experiments were undertaken because we became convinced after many years of clinical experience that despite perfect fixation, accurate reduction and adequately delayed weight bearing, non-union and aseptic necrosis developed in many cases.[3] Cleveland, Larsen[4] and many others have reported the same difficulty despite the use of many different types of fixation materials.

## METHOD

Fifty newly acquired mongrel dogs of various ages were operated under intravenous veterinary sodium pentothal anesthesia. Strict aseptic precautions were observed. The right hips were approached through lateral incisions; *the hip abductors were sectioned and the capsules were opened by* "T" shaped incisions. The ligamentum teres was sectioned in all but 2 cases. The femoral necks were osteotomized utilizing the Gigli saw and the heads were completely removed from the wounds. Extensive capsular stripping was carried out proximally. The heads were then replaced and accurately fixed by stainless steel screws under direct observation. The capsules and the soft tissues were closed without undue tension and the animals were returned to individual cages for postoperative care.

**Group One—Control Group Five Animals.** The procedures described above were carried out. All the animals developed aseptic necrosis and non-

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## METHOD

The data were obtained by review of the case records of all patients with a diagnosis of hip fracture seen at the University Hospitals during the past 15 years Blood type information and diagnosis were the 2 main factors in the selection of cases Patients over 60 yr of age with intra and extra capsular fractures resulting from minimal trauma were accepted Younger patients with similar fractures or patients with fractures resulting from severe trauma were not The significance of differences in the blood type frequencies of the patient and control groups was tested by the Chi square and difference in percentages methods Blood donors all friends or relatives of the patients from the same geographical area with similar genetic anthropologic and ethnic backgrounds were use as controls

The blood type frequencies of the patient group were also compared with those of a second group of patients of all ages who had incurred various kinds of fractures incident to severe trauma To control the age factor the blood type frequencies of patients over 60 yr of age receiving consecutive blood transfusions were recorded and compared with those of patients with hip fractures

In examining the significance of the observed differences 11 different combinations of the 4 blood types are possible Only the results of the O A and O A B AB comparisons are reported The other comparisons were made but added nothing of importance to the analysis as has been true in general in these researches A detailed description of the methods employed in collecting and analyzing the data appears elsewhere [2, 3]

## RESULTS

In Table 1 are recorded the numbers and percentages of the controls and hip fracture patients with each blood type By examining the differences in the percentage of the controls and patients for each blood type it will be noted that the most striking finding is the increase in blood type A and decrease in O in the patients lesser differences in percentage are noted for types A and AB When the Chi square analysis is made for the O A differences $02 < P < 05$ indicating significance whereas when the O A B AB differences are examined $10 < P$ indicating no significance The lack of significance for the second analysis is due to the minor differences in the B and AB frequencies

Inspection of the data in Table 1 suggests that the evidence for the blood group association is stronger in men than in women However no evidence of statistical significance $10 < P$ was found for the differences occurring between men and women Likewise none was found when the blood type frequencies of the male and female patients were separately compared with the controls

In Table 2 are recorded the data obtained from patients with other kinds of fractures and data from patients 60 yr of age and older receiving transfusions Comparing the blood type frequencies of hip fracture and other fracture patients $10 < P$ for the O A and O A B AB comparisons This was also true when the data obtained from men and women were separately examined For the differences observed between the hip fracture and transfused patients $02 < P < 05$ for the O A comparison and $10 < P$ for the

necrosis can be prevented and creeping substitution so necessary for the healing of fractures can be speeded up

Further work in which radioactive tracers are used to measure circulation accurately and provide us with an index with which we can prognosticate the future of the damaged bone will be carried out[5] The inferences that *may be drawn from our pilot study are interesting though speculative We* believe that transplants of the rectus muscle in humans will be feasible We hope we are one step nearer to the solution for the unsolved fracture

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# ON THE ETIOLOGICAL ROLE OF HEREDITY IN FRACTURES*

J A BUCKWALTER J H TURNER, L RATERMAN AND R T TIDRICK

During the past 3 years increasing numbers of investigations have been reported concerning the association of the ABO blood groups and disease Interest in this field was reawakened by the report of Aird Fraser Roberts and Bentall[1] in 1953 indicating a highly significant association between the ABO blood groups and gastric carcinoma in the population of Great Britain

The results of studies at the University of Iowa Hospitals[2] have in general confirmed those of the British authors and suggested associations in additional diseases In evaluating these data the blood type frequencies observed in the patient groups have been compared with those noted in a disease free sample of the population referred to as controls Differences between the blood type frequencies of the patients and controls have been examined for statistical significance For the purpose of obtaining additional control data patients with hip fractures were studied The blood type frequencies differed significantly from those of the controls which was not expected This is a preliminary report of the investigations arising from this chance observation

*From the Department of Surgery College of Medicine and the Department of Mathematics and Astronomy State University of Iowa Iowa City Iowa Statistical analysis by L A Knowler

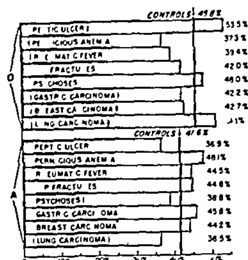



Fig 1 Blood type O and A percentages of controls (blood donors) and patients

the differences between the patients and controls when the O A B AB comparison was made

The data obtained from patients with other fractures were of interest because of the close agreement of the blood type frequencies with those of the controls thus differing from the hip fracture patients However be cause of the small number of patients in the former group no significance was found for these differences Likewise no significance was found for the differences in the blood type frequencies of the male and female hip frac ture patients although inspection of the data suggests the possibility of a difference Sample size may or may not be responsible for the absence of significance

Information available with regard to blood type frequencies in persons 60 yr and older is sparse and does not permit a categorical statement to be made that they are the same as those noted in younger persons of the same population Since blood donors are all younger persons they do not provide an answer to this question Patients receiving blood transfusions provide a control for the age factor which is admittedly a poor one since their dis orders also may be associated with alteration of blood type frequency Per haps the best control is provided by comparing the frequencies noted in the hip fracture patients with other patient groups When this is done highly significant differences between patient groups are noted In Figure 1 note the differences in blood type O and A frequencies for the various patient groups thus far studied comparing the blood type frequencies of hip fracture and peptic ulcer patients $P < 001$

Causality has as yet not been proved for any of the demonstrated ABO blood group disease associations However, with the accumulation of similar data from various areas of the world for different population groups indi cating associations for increasing numbers of diseases an as yet not under stood causal relationship related to heredity becomes more an anthropo logical or specious one less likely Senile osteoporosis played a causative role of varying importance in the patients with fracture of the hip The possible relationships of the blood group substances to protein metabolism endocrine function and other physiologic processes giving rise to defective bone matrix are at this time matters of sheer speculation

As suggested earlier life expectancy has not been correlated with the

*Table 1 Number and percentage of hip fracture patients and controls (blood donors) with each blood type, and difference in the blood type percentages of the patient and control (blood donor) groups*

| BLOOD TYPE | PATIENTS | | | | | | | | | CONTROLS | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | MEN | | DIFF IN % FROM CONTROLS | WOMEN | | DIFF IN % FROM CONTROLS | TOTAL | | DIFF IN % FROM CONTROLS | | |
| | NO | % | | NO | % | | NO | % | | NO | % |
| O | 130 | 39.6 | −6.2 | 280 | 42.9 | −2.9 | 410 | 41.8 | −4.0 | 2892 | 45.8 |
| A | 149 | 45.4 | +3.8 | 292 | 44.7 | +3.1 | 441 | 45.0 | +3.4 | 2625 | 41.6 |
| B | 35 | 10.7 | +1.7 | 60 | 9.2 | +0.2 | 95 | 9.7 | +0.7 | 570 | 9.0 |
| AB | 14 | 4.3 | +0.7 | 21 | 3.2 | −0.4 | 35 | 3.5 | −0.1 | 226 | 3.6 |
| Totals | 328 | 100.0 | 0.0 | 653 | 100.0 | 0.0 | 981 | 100.0 | 0.0 | 6313 | 100.0 |

*Table 2 Blood types and blood type percentages of patients with other fractures and of transfused patients 60 yr of age and older differences in the blood type percentages from those of hip fracture patients and the controls (blood donors)*

| BLOOD TYPE | OTHER FRACTURE PATIENTS | | | | TRANSFUSED PATIENTS | | | |
|---|---|---|---|---|---|---|---|---|
| | | | DIFFERENCE IN % FROM | | | | DIFFERENCE IN % FROM | |
| | NO | % | HIP FRACTURE PATIENTS | CONTROLS | NO | % | HIP FRACTURE PATIENTS | CONTROLS |
| O | 112 | 44.4 | +2.6 | −1.4 | 810 | 45.4 | +3.6 | −0.4 |
| A | 103 | 40.9 | −4.1 | −0.7 | 730 | 41.0 | −4.0 | −0.6 |
| B | 26 | 10.3 | +0.6 | +1.3 | 177 | 10.0 | +0.3 | +1.0 |
| AB | 11 | 4.4 | +0.9 | +0.8 | 64 | 3.6 | +0.1 | 0.0 |
| Totals | 252 | 100.0 | 0.0 | 0.0 | 1781 | 100.0 | 0.0 | 0.0 |

O A B AB No significance was found on examining the data by sex No significance was found for the differences in the blood type frequencies of the patients with other fractures transfused patients and the controls

The Rh blood type was recorded in 804 of the patients with hip fractures The frequencies noted in the total patient group in the patients with type O A B and AB blood and in male and female patients did not differ significantly from the Rh frequencies observed in the controls

## DISCUSSION

The evidence presented suggests an association between the ABO blood groups and hip fractures This is characterized by a significantly higher frequency of blood type A and lower frequency of blood type O in patients with these fractures or there was a significantly higher incidence of this lesion in persons with blood type A and lower in those with blood type O The incidence in persons with blood types B and AB did not differ from the expected as indicated by the failure to find statistical significance for

# *Plastic Surgery*

## MODIFICATION OF HOST HOMOGRAFT TISSUE INTERACTIONS IN SEPARATED PARABIONTS*

RICHARD H. ANDRESEN, CLARENCE W. MONROE, DOROTHY A. MADDEN AND GEORGE M. HASS

Previous studies of rabbits united in parabiosis by the aural method showed normal sequences of healing along the line of surgical union. Three to 4 days following anastomosis, a cross circulation was established between parabionts. Epithelial continuity was attained and a mutual deposition of collagen and reticulum occurred in a manner so as to tightly adhere the homologous surfaces together. In 7 to 10 days after anastomosis a characteristic inflammatory reaction developed which appeared to interfere with the dermal continuity and the cross circulation. In spite of the intense inflammatory reaction and the ischemia along the line of anastomosis, the aural junctions remained well apposed for as long as 5 mo in some experiments. Apparently the mutually deposited collagenous matrix was resistant to the autolytic mechanisms operating in most inflammatory processes.[1]

Additional studies showed that if parabionts once separated from one another were rejoined, cross circulation was rarely established and the aural junctions usually separated spontaneously by the 5th or 6th day. This observation raised a question as to the nature of difference between parabionts following the initial period of parabiosis.

In an attempt to further characterize the effect of parabiosis the following experiments were designed. It seemed reasonable to assume that such differences might become apparent through a critical analysis of the host graft tissue interactions under aseptic circumstances, such as is the case with musculofascial grafts.

### METHOD

Thirty pairs of New Zealand rabbits were surgically united (aural method) for 13 to 15 days and then separated by amputation of the joined ears under 1 per cent procaine solution. Ten to 21 days after separation 15 pairs were reunited in a similar manner and again separated. The 15 remaining pairs were united once and separated after 10 to 21 days.

At intervals of 6 to 90 days following separation, grafts of erector spinae muscle measuring 2.0 × 1.5 × 0.5 cm with attached fascia were resected under sterile precautions and cross transplanted between respective parabionts. Control autografts were also made. Two weeks following transplantation, the animals were sacrificed via cisterna puncture with 1 per cent procaine solution. The skin was removed from the backs and the animals

*Rush Laboratories of Pathology and Surgical Research, Presbyterian Hospital, Chicago, Ill. Supported by grant H 1630 from the National Heart Institute, U.S.P.H.S.

ABO blood groups It is conceivable that those persons with group A have shorter life expectancy than group O and both shorter than group B These matters will have to be settled by extensive observations and careful statistical evaluation

Confirmation or rejection of the suggested association and the answers to the questions concerning sex and age should be provided by the collection of additional data However, the answer to the question concerning causality will be more difficult to obtain In addition to similar data collected from different racial and population groups, appropriate basic investigations designed by geneticists biochemists, endocrinologists enzymologists and clinicians will be required


SUMMARY

The ABO blood type frequencies of patients with hip fractures have been compared *with those of blood donors with patients with miscellaneous* fractures and with transfused patients 60 yr of age and older Evidence of statistical significance was found suggesting an association between the ABO blood groups and hip fractures characterized by an increased incidence of this injury in persons with blood type A These findings suggest a possible role for heredity in the etiology of hip fractures



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Fig 2 This is a low power photomicrograph (Magnification ×10) of a postparabiotic musculofascial cross homograft 2 wks of age This graft is similar to that shown in Fig 1 with the exception that the fascia and area beneath contain a peculiar angiomatous pattern of patent vascular channels In spite of this rich vascularization collagen deposition and fibroblastic proliferation are curtailed Inflammatory cell infiltrate and angiitis are not observed This represents the second form of postparabiotic host homograft tissue interaction and is found in one half of the group of separated rabbit parabionts

the graft remained delayed and there was no tendency to elicit the characteristic inflammation usually seen in homografts (Fig 2)

DISCUSSION

The present studies have shown that the host homograft tissue interaction in the postparabiotic period is highly specific and readily reproducible between parabiotic partners for as long as 90 days This type of host graft tissue interaction is different from those previously described[2] It appears to resemble more closely the host graft tissue interactions obtained when homologous musculofascial grafts are treated at low temperatures or with x irradiation *in vitro* prior to transplantation

In order to determine whether this modification was applicable to other types of homografts a series of skin homografts was made between separated parabionts These experiments have disclosed that the skin homografts made between separated parabionts in the postparabiotic period are remarkably well tolerated The grafts do not slough away as is customary for skin homografts in a period of about 2 wks On the contrary the grafts persist though they slowly shrink in dimensions finally disappearing over a period of many weeks The appearance of these grafts at the end of 9 weeks is shown in Figure 3



Fig 3 This is an illustration (Natural Size) showing 2 skin cross homografts in a separated rabbit parabiont 9 wks after transplantation Note that the grafts after this period of time remain sharply demarcated and are well healed in place The grafts appear to be well tolerated by the host

placed in 10 per cent formalin solution Following fixation the grafts were sectioned in a plane perpendicular to the spinal column The tissues were embedded in paraffin cut and stained with hematoxylin and eosin

RESULTS

Gross examination of the musculofascial grafts at 2 wks of age showed that they were well healed in place A bursal space had formed over both types of grafts The autologous musculofascial grafts showed a red heavy pannus of vascularized granulation tissue which had grown over the fascia of the grafts On the other hand, the parabiotic homografts showed gradations from a pearly white appearance to one showing pink streaking indicative of the varying degrees of vascularization of the pannus by the host

Microscopic examination of the autologous musculofascial grafts in separated parabionts at 2 wks of age revealed the customary pattern of tissue reaction which consisted of orderly sequences of degeneration and organization in the absence of inflammation

Microscopic examination of the parabiotic homografts cross transplanted between respective parabionts showed 2 types of host graft tissue interactions which were about equally distributed between the 2 groups of separated parabionts The first type of reaction was characterized by an avascular collagenous encapsulation of the graft There was delayed absorption of the graft though it was well healed in place The fascia of the graft remained normally cellular and was covered by an avascular pannus of mesenchyme derived from the host The muscle bundles remained in close approximation to the fascia so that absorption was taking place principally from the lateral and inferior aspects of the graft Inflammation was not observed although the muscular tissues of the host adjacent to the graft often contained foci of plasma cells (Fig 1)

The second type of tissue reaction in separated parabionts was similar to the first except that vascular penetration did occur This consisted of the development of a peculiar angiomatous pattern of patent blood vessels beneath the fascia of the graft In spite of the intense vascularization collagen deposition and fibroblastic activity did not appear Also absorption of



Fig 1 This is a low power photomicrograph (Magnification ×40) of a postparabiotic musculofascial cross homograft 2 wks of age The empty space above the tissue represents the bursal space which usually forms above all types of musculofascial grafts in rabbits The floor of the bursal space is formed by a delicate pannus of avascular mesenchymal tissue which has originated from the host Beneath the pannus is the fascia of the graft which is somewhat thickened due to retraction The muscle bundles of the graft remain well approximated to the overlying fascia The architecture of the graft is unchanged This host homograft tissue interaction represents one form encountered in parabiotic partners during the postparabiotic period

cific antibodies. Since the properdin system is a new and unexplored phase of the immune response, it appeared to offer a feasible field for the study of transplantation of tissues. This was of particular interest since the properdin system can be altered by the administration of certain agents (Zymosan, dextrans, levans and other carbohydrate complexes) thus allowing controlled host properdin responses to the transplantation of tissues.

To date the best evidence for failure of renal homotransplants appears to be a function of the host's immune response, although a specific blood born anti-tissue antibody has not yet been detected by standard serological techniques.[4] It has been postulated that suppression of this immune response would lead to successful homografts and many efforts have been directed towards this goal in the past.[5,6,7]

Therefore renal homotransplantation was undertaken to see what effect the depression of the properdin system would have on survival of homotransplants.

METHOD

The experimental laboratory animals utilized in this study were healthy mongrel dogs. The serum from 10 cc. of clotted blood was assayed for initial properdin levels in each of 19 animals. The properdin titers were determined by the standard technique described by Pillemer[1] and performed in his laboratory. Animals with titers above 2 units/ml. were suitable for Zymosan administration. (Zymosan is a suspension of insoluble cell wall residue from yeast.) The LD$_{50}$ was approximated in a preliminary group of 6 dogs by the intravenous administration of Zymosan suspension.

The animals were anesthetized with i.v. sodium pentobarbital 30 mg./kg. and then Zymosan was injected intravenously in dosages varying from 5 to 25 mg./kg. body weight. In 2 animals renal transplantation from donor dogs was done immediately. In 6 animals the Zymosan was administered 24 hr. prior to transplantation (Fig. 1).

After suitable exposure of the carotid and jugular vessels the carotid artery of the host was sutured to the renal artery of the donor and the jugular vein to the renal vein with 5-0 silk. The ureter was then brought out through a stab wound in the neck. A suitable pocket was formed in the neck for the donor kidney which was fixed *in situ* by sutures to prevent

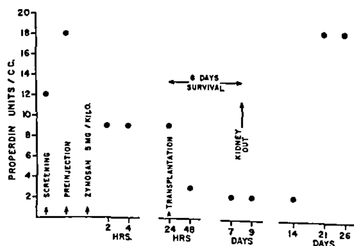



Fig. 1. (U 7) Showing usual time relationship of properdin levels, Zymosan administration and renal homotransplantation. Transplantation done 24 hr. after Zymosan. Properdin level low for about 14 days. Graft survived 8 days during period of low properdin levels.

SUMMARY

Rabbits were united in parabiosis by the aural method for varying periods of time and then separated From 6 to 90 days following separation musculofascial grafts were cross transplanted between specific parabiotic partners Two weeks after transplantation the grafts were prepared for microscopic study This revealed 2 types of host graft tissue interactions The first type consisted of an avascular collagenous encapsulation of the graft The architecture of the graft remained unaltered Inflammation did not occur The second type of host homograft interaction in separated parabionts was similar to the first except that vascular penetration was conspicuous A curious angiomatous pattern of vascular channels developed beneath the fascia of the graft In spite of the intense vascularization collagen deposition and fibroblastic proliferation did not take place

Other tissues such as skin cross homografts were studied in the post parabotic period Skin homografts under these conditions were well tolerated by the hosts for extended periods of time A method has been described by which the inflammatory aspects of the host homograft tissue interaction between 2 animals of the same species has been entirely eliminated

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# PRELIMINARY EXPERIENCE WITH PROPERDIN AND RENAL HOMOGRAFTS*

CHARLES A HUBAY LESTER PERSKY AND WILLIAM D HOLDEN

The properdin system described by Pillemer[1 2 3] and associates has been shown to be an integral part of the natural defense mechanism of blood Properdin has been felt to be separate and distinct from immune mechanisms involving antibody antigen reactions This serum protein is a euglobulin with a molecular weight 8 times that of gamma globulin and represents not more than 0 03 per cent of the total proteins It is dependent for its action upon complement and Mg ++ and is active against certain bacteria and viruses and lyses certain abnormal erythrocytes in the absence of spe

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*Table 1 Summary of results of renal homotransplantation in 8 experimental animals*

| ANIMAL | U6 | U7 | U35 | U11 | U18 | U19 | U59 | U61 |
|---|---|---|---|---|---|---|---|---|
| ZYMOSAN MG/KG | 5 | 5 | 25 | 5 | 10 | 10 | 5 | 5 |
| TIME OF TRANSPLANT | 24 hrs | 24 hrs | immediate | 24 hrs | 24 hrs | immediate | 24 hrs | 24 hrs |
| GRAFT SURVIVAL | 7 days | 8 days | 1 day | 8 days | 1 day | 2 days | 8 days | 8 days |
| INFECTION | + | + | ++++ | +++ | ++++ | +++ | +++ | +++ |

*Table 2 Properdin levels in experimental animals before and after Zymosan administration Note return to normal levels in animals surviving 2 or more wks*

| ANIMAL PROPERDIN TITERS | U6 | U7 | U35 | U11 | U18 | U19 | U59 | U61 |
|---|---|---|---|---|---|---|---|---|
| Preliminary | 12 | 12 | 16 | 9 | 18 | 21 | 12 | 12 |
| Preinjection | 18 | 18 | 12 | 5 | 10 | 18 | 2 | 2 |
| 1 hr | 18 | | 2 | 1 | 10 | 12 | 3 | 2 |
| 2 hr | 18 | 9 | 2 | 5 | 2 | 6 | 1 | 2 |
| 4 hr | 18 | 9 | 2 | 5 | 2 | | 4 | 2 |
| 24 hr | 18 | 9 | 2 | 2 | 2 | 1 | 2 | 2 |
| 48 hr | 6 | 3 | | | | | | |
| 72 hr | 3 | | 2 | | 2 | 1 | 2 | 2 |
| 1 wk | 3 | 3 | 2 | | | 18 | 2 | 2 |
| 9 days | 3 | 3 | | | | | | |
| 10 days | 3 | | | 2 | | | 2 | 2 |
| 2 wk | 21 | 3 | 2 | | | 20 | | 15 |
| 3 wk | 21 | 18 | | | | | | |
| 26 days | 18 | 18 | | | | | | |
| 4 wk | 18 | | | | | | | |
| Fate | Sac | Sac | Died | Sac | Died | Sac | Sac | Sac |

It should be pointed out that control studies of the effect of Zymosan on the properdin system in dogs would have been desirable This was not feasible however owing to the small number of dogs suitable for Zymosan administration (22 5 per cent of dogs screened)

Although these preliminary experiences with renal homografts and the properdin system were not successful we feel that this type of study should be continued with more suitable dogs maintained under optimum conditions or alternatively with another species of experimental animal whose properdin level is less labile and who is innately more resistant

SUMMARY

The properdin system was explored in dogs to determine its role in the response of recipient animals to renal homotransplants When the properdin system was artificially depressed homotransplantation was not favorably

The authors wish to express their appreciation to Dr Louis Pillemer whose counsel and laboratory aid made this study possible and to Dr Simon Koletsky for microscopic examination of the transplanted kidneys

rotation and kinking of vessels The subcutaneous tissues and skin were then closed with 4–0 silk A small polyethylene catheter was then inserted into the ureter to the renal pelvis and sutured to the skin This allowed daily saline irrigations to remove clots and insure against kinking of the ureter

Additional properdin titers were drawn at periodic intervals after the administration of Zymosan

### RESULTS AND DISCUSSION

Forty nine animals were screened and initial properdin titers obtained Of these animals only 11 had titers which were deemed adequate for Zymosan administration For technical reasons 3 of these animals could not be used This over all percentage of 22 5 suitable levels reflects the general *undesirability of using mongrel dogs in experiments involving the properdin* system The titers may be low owing to chronic infestations and infections which are so often found in pound obtained dogs This observation has been substantiated by the experience of Fine[8] and Michaelson (personal communication to Pillemer) has found that the proper care of inbred beagle dogs result in consistently normal properdin titers An alternative would be the employment of a different experimental animal In a study such as *renal homotransplantation* however *the size of the renal vessels in smaller* animals precludes their use routinely

In the preliminary determinations of the approximate LD$_{50}$ it was found that dosages over 25 mg /kg of Zymosan saline suspension intravenously resulted in the death of the animal within 24 hr The administration of Zymosan in these doses was accompanied by vomiting severe tenesmus bloody diarrhea wheezing respirations and rapid death Therefore Zymosan *dosages of 5 to 10 mg /kg were used giving satisfactory depression* of properdin levels

The length of survival of the homografts is illustrated in Table 1 Survival varied from 24 hr to 8 days The death of the kidney was attended by diminution of urine output and hematuria Microscopic examination of the kidneys showed typically severe pyelonephritis

Properdin titers were depressed by administration of Zymosan and these are shown in Table 2 One of the difficulties encountered in this study was the spontaneous fall of the properdin level between the screening and the subsequent Zymosan injection and operative procedure This period was approximately 2 wks during which time the animal was maintained under routine kennel care

Death in 2 animals (U35 48) appeared to be from overwhelming septicemia despite postoperative penicillin and streptomycin therapy The remaining animals were given oral neomycin 1/0 days for 2 days prior to transplantation and received postoperative penicillin streptomycin therapy Nevertheless these animals also developed non fatal infections at the trans plantation site

The relationship between the hypoproperdinemia and the bacterial in fections noted in these animals has been substantiated by the work of Wardlaw and Pillemer[9] who demonstrated that the removal of properdin from serum also removes bactericidal activity The addition of properdin to *properdin-deficient serum restored bactericidal activity*

METHOD

Five pairs of mongrel dogs were used, maintaining the same donor-recipient relationship throughout. Under nembutal anesthesia a 10 × 5 cm recipient area on a shaved portion of the chest wall was outlined with a scalpel. The skin was removed down to the level of the panniculus carnosus by blunt dissection requiring fairly strong traction. In this manner the plane of cleavage was easily found and bleeding minimized. Different recipient areas were used for each operation. Split thickness grafts of donor skin were cut with a thin bladed knife, several pieces being used to cover the area. Control autografts were applied in a number of instances. The grafts were covered by fine mesh gauze and fixed in position by cotton waste and adhesive tape, the whole area being protected by a calico jacket.

The grafts were inspected daily from the fourth day, the survival time being judged by gross appearance. Serial color photographs were invaluable in retrospective assessment of rejection. Serial biopsies were taken where practicable. No dressing was required after the seventh day. The time interval between grafts varied from 7 to 55 days and the total number of operations for each pair of animals ranged between 3 and 10.

RESULTS

At the first dressing after the first operation the graft was well vascularized in each instance. There was slight epithelial outgrowth from the edges of the graft. Subsequently the whole area became completely epithelialized, the graft becoming supple (Fig 1a and b). The first sign of rejection was a color change of the graft from normal to dark red and finally to black. These changes occurred over a period of 24 to 48 hr, followed rapidly by complete sloughing of the graft. Re-epithelialization occurred from surrounding host epithelium or from the control autograft, leaving this recipient bed contracted.

After the later serial operations the appearance of the graft on first inspection was very different. In some instances the soft, swollen and moist graft demonstrated no evidence of epithelial outgrowth at its margins and could be detached easily from its bed (Fig 2a and b); in others only islands of the transplanted skin were present and the remaining area consisted of friable granulations which bled readily, evidence of a marked homograft




Fig 1 Appearance of 1st set split thickness skin homograft (a) at operation and (b) on the 5th day showing survival and epithelial proliferation (Dog #3)

influenced It is pointed out that mongrel dogs under average kennel conditions are unsuitable for this type of experiment owing to the high percentage with initially low properdin titers


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# A STUDY OF CONSECUTIVE SKIN HOMOGRAFTS IN THE DOG*

LEWIS THOMAS AND JOSEPH E MURRAY

An attempt has been made to modify the homograft reaction in the dog by the application of consecutive skin grafts from the same donor to the same recipient Medawar[1] using rabbits demonstrated that the second homograft from the same donor is rejected in a shorter time than the first and postulated an antigen antibody reaction as the basis of the rejection This has been the current working hypothesis for most studies of homotransplantation *An increased percentage of permanent survival of homografts* after a series of skin grafts on heterozygous CFW mice has been described by Werder and Hardin presumably by overwhelming the immune reaction by the repetitive doses of antigen[3] The present study is an attempt to apply this principle of immunoparalysis to an animal with genetic and immunologic characteristics more nearly resembling man

As the homograft problem in man is being more fully investigated and understood it appears that patients with chronic uremia can tolerate homografts of skin and kidney longer than normal recipients If a further lowering of the immune response can be obtained in such recipients by serial applications of antigen the clinical usefulness of renal homotransplants might be expanded

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*From the Surgical Service Peter Bent Brigham Hospital and the Laboratory for Surgical Research Harvard Medical School Boston Mass Supported by a grant from the United States Public Health Service The assistance of the Morgan Williams Request Fund University of Wales is also gratefully acknowledged

## METHOD

Five pairs of mongrel dogs were used, maintaining the same donor-recipient relationship throughout. Under nembutal anesthesia a 10 × 5 cm recipient area on a shaved portion of the chest wall was outlined with a scalpel. The skin was removed down to the layer of the panniculus carnosus by blunt dissection requiring fairly strong traction. In this manner the plane of cleavage was easily found and bleeding minimized. Different recipient areas were used for each operation. Split thickness grafts of donor skin were cut with a thin bladed knife, several pieces being used to cover the area. Control autografts were applied in a number of instances. The grafts were covered by fine mesh gauze and fixed in position by cotton waste and adhesive tape, the whole area being protected by a calico jacket.

The grafts were inspected daily from the fourth day, the survival time being judged by gross appearance. Serial color photographs were invaluable in retrospective assessment of rejection. Serial biopsies were taken where practicable. No dressing was required after the seventh day. The time interval between grafts varied from 7 to 53 days and the total number of operations for each pair of animals ranged between 3 and 10.

## RESULTS

At the first dressing after the first operation the graft was well vascularized in each instance. There was slight epithelial outgrowth from the edges of the graft. Subsequently the whole area became completely epithelialized, the graft becoming supple (Fig. 1a and b). The first sign of rejection was a color change of the graft from normal to dark red and finally to black. These changes occurred over a period of 24 to 48 hr, followed rapidly by complete sloughing of the graft. Re-epithelialization occurred from surrounding host epithelium or from the control autograft, leaving this recipient bed contracted.

After the later serial operations the appearance of the graft on first inspection was very different. In some instances the soft, swollen and moist graft demonstrated no evidence of epithelial outgrowth at its margins and could be detached easily from its bed (Fig. 2a and b); in others only islands of the transplanted skin were present and the remaining area consisted of friable granulations which bled readily, evidence of a marked homograft




Fig. 1. Appearance of 1st set split thickness skin homograft (a) at operation and (b) on the 5th day showing survival and epithelial proliferation. (Dog #3)

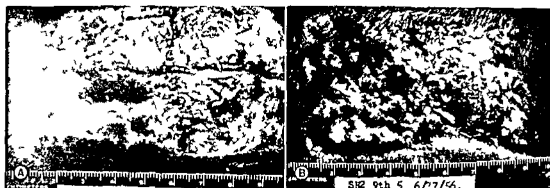



Fig 2 (a) 8th set homograft on the 6th post operative day (Dog #2) (b) 9th set homograft on the 5th post operative day (Dog #2) Note edematous indolent appearance of grafts (Contrast to Figure 1b)

reaction (Fig 3) Occasionally infection secondary to rejection made assessment of survival difficult Most autografts survived and finally increased in size to cover the defect left by the sloughing homograft In 2 instances autograft failure was due to infection

The survival time of the first set graft ranged from 6 to 15 days with each consecutive set the time became progressively shorter to about 4 days on the third set With these later grafts there was never any epithelial proliferation edema was prominent and the area was hyperemic further evidence of the homograft reaction At no time even after 10 consecutive grafts was there any evidence of prolonged survival of the skin transplanted Instead each graft was rejected in a shorter time than the initial graft of the series (Table 1) Prolongation of the interval between grafts increased the period of survival to a slight degree In Dog #3 after a 45 day interval graft survival time increased from 4 days to 6 but still did not approach the 9 day survival period of the first set graft (Fig 4) This slight increase in survival time after the 45 day interval may not appear significant in time but the gross appearance of the graft indicated a less severe homograft reaction The outline and substance of the graft persisted whereas the earlier graft was completely destroyed at the fourth day All data are tabulated in Table 1 In Figure 4 the data for dogs #2 33 and 38 are charted in order to indicate more graphically the variations in survival time related to the intervals between grafting



Fig 3 2nd set homograft at 7th post operative day Small areas of homograft remain but are black and will slough in 24 to 48 hours An autograft is surviving at the right margin (Dog #9)

*Table 1 Survival times of serial skin homografts in dogs*

| OPERATION | DOG 1 INTERVAL BETWEEN OPERATIONS | DOG 1 DAYS OF GRAFT SURVIVAL | DOG 2 INTERVAL BETWEEN OPERATIONS | DOG 2 DAYS OF GRAFT SURVIVAL | DOG 3 INTERVAL BETWEEN OPERATIONS | DOG 3 DAYS OF GRAFT SURVIVAL | DOG 8 INTERVAL BETWEEN OPERATIONS | DOG 8 DAYS OF GRAFT SURVIVAL | DOG 9 INTERVAL BETWEEN OPERATIONS | DOG 9 DAYS OF GRAFT SURVIVAL |
|---|---|---|---|---|---|---|---|---|---|---|
| 1st | — | 8 | — | [illegible] | — | 9 | — | 6 | — | [illegible] |
| 2nd | [illegible] | 6 | 23 | 7 | 11 | [illegible] | 20 | [illegible] | [illegible] | [illegible] |
| 3rd | 11 | 1 | [illegible] | | 7 | 1 | 16 | 4 | [illegible] | 4 |
| 4th | 13 | DIED | 11 | 1 | 17 | 1 | 10 | 6 | | |
| 5th | | | 7 | 4 | 7 | 1 | 30 | 1 | | |
| 6th | | | 1 | 5 | 11 | 4 | 10 | 4 | | |
| 7th | | | 17 | 6 | 13 | 4 | | | | |
| 8th | | | [illegible] | 7 | [illegible] | 6 | | | | |
| 9th | | | 36 | 5 | 11 | [illegible] | | | | |
| 10th | | | | | 37 | [illegible] | | | | |

## DISCUSSION

This investigation has been one phase of a clinical and experimental study of the homotransplantation of kidney and skin. The dog rather than inbred strains of mice and rabbits has been selected as the experimental animal because their genetic and immunologic characteristics more nearly resemble man and make results more useful. Most experimental data derived from the lower animal probably should be tested in the dog prior to clinical application.

In order to overwhelm the immune mechanism the maximum antigenic response is desirable. Therefore large areas of skin were grafted because Medawar[4] has demonstrated that smaller homografts possess a lower dose of antigen and therefore survive longer than large ones.

The results seem definite: there is no increase in the survival of skin homografts in the dog following the application of serial homografts. There is no evidence that the immune mechanism is overwhelmed or the antibody response lowered by repetitive doses of antigen.

SERIAL SKIN HOMOGRAFTS

Dog 2

Dog 3

Dog 8

Fig 4. Average survival times of skin homografts for dogs #2, #3, #8 indicating accelerated rejection after 1st operation.
Note the relative increase of survival if interval between operations is prolonged.

There is evidence that the accelerated rejection of a second set homograft is not permanent. It can be modified by the subsequent plan of experimentation. After a lapse of 15 days the immune reaction diminishes as evidenced by a slight prolongation of survival of the graft. However in no animal did any subsequent graft, even after a 55 day interval, survive longer than the original graft. This may contrast with the observations in mice of Lehrfeld, Taylor and Converse that second homografts applied 60 days after the first survived as a primary graft and did not demonstrate the second set phenomenon. Either there is a species difference or a longer interval is required to eradicate the heightened immunity in the dogs. In our present series more grafts will be applied at longer intervals to determine the length of survival of this accelerated reaction.

### CONCLUSIONS

The results support the immune mechanism theory of homograft rejection, the state of heightened immunity being shown by the more rapid rejection of each set of consecutive homografts. The homograft survival was not prolonged by serial homografting. This method does not seem suitable for clinical trial.

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## THE FATE OF THE SKIN HOMOGRAFT IN THE CHRONICALLY UREMIC PATIENT*

NATHAN P COUCH, JOSEPH E MURRAY, GUSTAVE J DAMMIN AND LEWIS P THOMAS

Because an immune response underlies rejection of tissue homotransplants in man and the experimental animal, studies of both naturally occurring and artificial means of suppressing immune mechanisms have special meaning for all surgeons concerned with tissue grafting.

*Aided by the United States Public Health Service, John A Hartford Foundation and the Medical Research and Development Board, Office of the Surgeon General, Department of the Army
With the assistance of Dr John Merrill, Dr Warren Guild and the Kidney Laboratory of the Peter Bent Brigham Hospital

*Homovital* (as opposed to *homostructural*) tissue transplantation has proven value in chronic renal failure in kidney transplantation between identical twins where the recipient has chronic uremia virtual cure can be achieved[8] Even when the chronically uremic recipient receives a renal homograft from an unrelated donor homotransplant function is sometimes maintained for 5 to 25 wks In these patients with widely varying types of renal disease chronic uremia is the outstanding common denominator In the normal dog renal homograft function usually fails before 1 week[7] and in man where renal homografts have been performed for acute rather than chronic renal failure function ends by 3 weeks[10] In some chronically uremic patients then renal homograft survival appears to be prolonged Since antigenic kinship between skin and kidney has been demonstrated in the dog it seemed plausible and worthy of experimental trial that homografts of skin might also enjoy extended survival in the presence of chronic uremia

The course of vascularization and rejection of the human skin homograft in the normal recipient is constant with eschar formation by day 15 and sloughing by day 20 leaving a dermal pad in the host bed[2] Gibson and Medawar[6] observed that epidermal degeneration in skin homografts was grossly complete by 23 days while in contrast the dermal collagen persisted for at least 36 days and underwent destruction before 60 days

Our objective in the present study was to determine whether there is in fact prolonged skin homograft survival in patients with chronic renal failure

## METHOD

Ten patients were used These included 6 patients (Group A) with chronic uremia due to differing forms of renal disease chronic pyelonephritis chronic glomerulonephritis and polycystic kidneys The durations of uremia varied from 1 mo to 6 yr while ages ranged from 20 to 62 yr Skin donors were obtained without attempt to secure blood group compatibility between donor and recipient In 2 cases grafting was between opposite sexes Three patients received more than 1 homograft A total of 4 homografts came from donors on long term cortisone treatment 2 from other chronic uremic patients and 5 from metabolically normal persons Three other patients (Group B) also uremic received similar homografts from their healthy identical twins Finally a control male adult received 2 normal skin homografts

Homografts and control autografts were placed on the recipients upper extremities The homografts were of split thickness the autografts were of full thickness and the recipient beds were subcutaneous tissues All grafts were 2 to 3 cm square and were fitted and sutured to adjacent recipient skin Gross observations and photographs were made at frequent intervals beginning no later than the seventh day after grafting

Most grafts were biopsied once only the biopsy dates ranging from post grafting day 32 to day 115 Specimens were fixed in 10 per cent formalin processed through modified Bouin solution and stained with hemotoxylin eosin periodic acid Schiff Verhoeff van Gieson reticulum and Feulgen stains

Our histologic estimate of *epidermal* survival is still uncertain It is theoretically feasible to distinguish between donor and recipient epidermis when

There is evidence that the accelerated rejection of a second set homograft is not permanent It can be modified by the subsequent plan of experimentation After a lapse of 45 days the immune reaction diminishes as evidenced by a slight prolongation of survival of the graft However in no animal did any subsequent graft even after a 55 day interval survive longer than the original graft This may contrast with the observations in mice of Lehrfeld Taylor and Converse that second homografts applied 60 days after the first survived as a primary graft and did not demonstrate the second set phenomenon Either there is a species difference or a longer interval is required to eradicate the heightened immunity in the dogs In our present series more grafts will be applied at longer intervals to determine the length of survival of this accelerated reaction

CONCLUSIONS

The results support the immune mechanism theory of homograft rejection the state of heightened immunity being shown by the more rapid rejection of each set of consecutive homografts The homograft survival was not prolonged by serial homografting This method does not seem suitable for clinical trial

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uremic donors showed no consistent differences from the normal homografts. The homografts between identical twins (Group B) were all graded "excellent," while those placed on the normal control underwent frank rejection by 13 days and eventual scarring.

The results in the 6 chronic uremic patients and 1 identical twin are outlined in Table 1.

In Figure 1 two homografts undergoing rejection at day 13 in the control patient (Fig. 1a) are compared with the day 21 surviving homografts (Fig. 1b) in a uremic patient (M.S.). While the rejected homografts are dark brown, coarse, thickened, dry, wrinkled, and underlain by yellowish exudate, the surviving homografts retain normal pink color, normal texture, and normal rectangular shape without exudation.

In Figure 2 a perfectly surviving homograft between identical twins is compared histologically with a healed rejection site in a chronic uremic patient (from a later study) at day 12 (rejection completed at day 11), a surviving homograft in a uremic patient (J.T.) at day 33, and a second surviving homograft in another uremic patient (D.B.) at day 115. While the rejected graft site showed absence of rete pegs, almost complete loss of elastica, dense fibroplasia, and only scattered dermal appendages, the latter two grafts maintained fairly normal elastica pattern, intact collagen, minimal inflammation, and dermal appendages of surprisingly normal appearance.

## DISCUSSION

Although these studies are incomplete, survival of dermal elements appears to be prolonged, in varying degrees, in some chronic uremic patients. Our evidence for similar prolongation of epidermal survival is based on gross criteria. If the graft epidermis is slowly and insidiously replaced by ingrowth of host epidermis, the absence of the classical gross features of epidermal rejection in homografts of this size is still a notable departure from the normal.

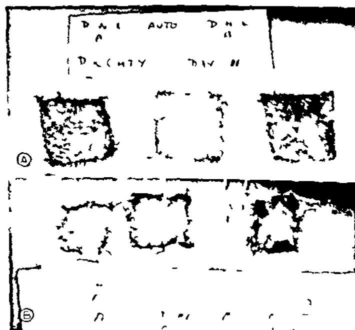



Fig. 1. (1a) From left to right: homograft, autograft and homograft on a normal control at day 11. Darkening, thickening and exudation are apparent and indicate early phase of rejection. (1b) From left to right: autograft, pure homograft, raw surface and homograft from a cortisone treated patient at day 21. Except for necrosis of cortex in one homograft around sutures, both homografts appear similar to autograft.

the 2 are of opposite sex by the chromocenter count method.[11] This technique depends on the fact that the female sex chromatin, which is larger than the male chromocenter and visible at the nuclear periphery, appears in a much higher percentage of epidermal cells in the female than in the male. In 2 such cases, the differential counts were unconvincing. (Methods for similar counts on whole cells garnered from epidermal scrapings and stained by cytological methods may be more satisfactory.)

Our evaluation of *dermal* survival depends on persistence of its appendages (hair follicles, sweat glands, smooth muscle, and sebaceous glands), the patterns of the elastica and collagen, the subepidermal (PAS positive) mucopolysaccharide, and the degree and type of inflammatory cell infiltration—all components separated from the recipient tissue by a sheet of dense scar.

RESULTS

Survival ratings were arbitrarily graded "poor," "fair," "good," and "excellent," since the survivals did not follow an all or none principle. Of the 11 homografts in the first 6 uremic patients (Group A), 4 were rated "poor," 2 "fair," and 5 "good." In all 7 grafts rated "fair" or better at no time was there gross evidence of rejection. The homografts from cortisone treated and

*Table 1. Survival of Skin Homografts*

| RECIPIENT | SURVIVAL OF SKIN HOMOGRAFTS DONOR | HOMOGRAFT | INTERVAL | SURVIVAL |
|---|---|---|---|---|
| MS 53 F A+ | RD M A+ | Pure | 32 d | Good |
| | RI M O | Cortisone | 32 d | Poor |
| JI 27 M O+ | LL 57 M A+ | Cortisone | 33 d | Good |
| | JN 22 M O+ | Pure | 33 d | Poor |
| | AK 62 M B+ | Uremic | 33 d | Infection |
| RH 23 M O+ | RH 23 M O+ | Pure | 37 d | Excellent |
| AK 62 M B+ | LL 57 M A+ | Cortisone | 57 d | Fair |
| | JN 22 M O+ | Pure | 57 d | Poor |
| | JI 27 M O+ | Uremic | 57 d | Fair |
| JW 20 M O+ | MS 59 F O+ | Pure | 61 d | Good |
| RM 26 F O+ | MM 19 F O+ | Cortisone | 115 d | Good |
| DB 33 M A+ | WL 52 M O+ | Pure | 115 d | Good |

All recipients are from Group A (see "Methods") except RH, who received a homograft from an identical twin and is included for comparison.

terms of tissue homografting as well as our constant need for testing the applicability of animal data to the human this method of altering the immune response is under further investigation in the chronically uremic human


## SUMMARY

Prolonged survival in varying degrees of skin homografts was observed in some patients with chronic renal failure This accords with the occasional finding of prolonged function of renal homotransplants in similar patients Studies of additional normal control recipients by the same methods are in progress

We have not found abnormalities in the various aspects of the immune mechanism so far investigated but additional factors including antigen antibody combinations and the properdin magnesium complement system are under scrutiny



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# THE EFFECT OF SKIN CYCLES ON THE SURVIVAL TIME OF SKIN HOMOGRAFTS IN MICE*

Ira M Dushoff and Peter Randall

Considerable experimental work has been done in an effort to extend the survival time of skin homografts Rodents have been chiefly used and despite use of inbred strains there has been notable variation in the reported experimental results both within single experiments and between different experiments making interpretation of the data quite difficult In all of this

* From the Harrison Department of Surgical Research School of Medicine University of Pennsylvania

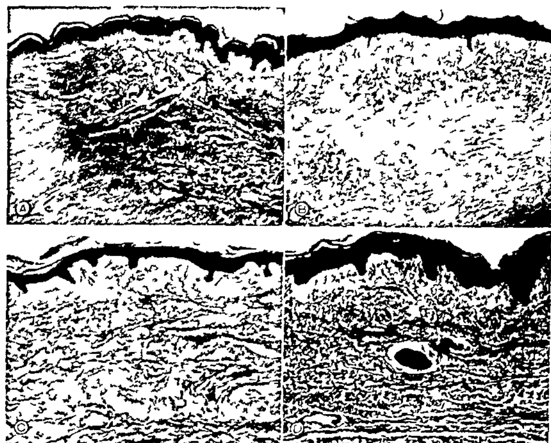


Fig 2 All Verhoeff van Gieson elastic tissue stains 2a—Case R H Homograft from identical twin donor at day 37 near junction of graft and recipient tissue Note normal rete pegs elastic tissue and collagen patterns and persistence of smooth muscle X128 2b—Case R I Site of rejected homograft in chronic uremia at 12 days Note absence of rete pegs monotonous collagen pattern and numerous capillaries Only a few strands of elastica remain in upper dermis X128 2c—Case J F Skin homograft in a chronically uremic patient at day 33 Note scattered dermal inflammatory cells good rete pegs and normal elastic tissue and collagen patterns X175 2d—Case D B Skin homograft in a chronically uremic patient at day 115 The rete pegs elastica and collagen patterns are preserved Graft recipient junction is at lower border X175

It must be stressed that completion of this study awaits evaluation of similar homografts in several additional normal control human recipients

The reason for this protracted survival are obscure Preliminary studies in several chronically uremic patients of serum gamma globulin, serum paper electrophoretic patterns skin tuberculin and Schick tests isoagglutinin titers and response to blood group specific substances have revealed no abnormalities Furthermore others have found no significant changes in complement levels attributable to acute uremia[1] or chronic uremia[7] Elevations of the alpha 2 globulin have been recently described in uremia[illegible]

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Considerable data from animal experiments indicate that immune responses may be altered by administration of various tissues prior to transplantation of the same or related tissues Blood elements have been extensively studied in this regard[8, illegible, illegible] Because of therapeutic implications in

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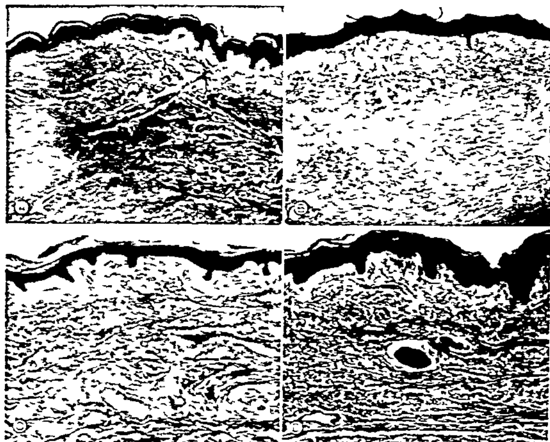


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Pillemer and Hinz[12] have demonstrated depressions of properdin levels in a small number of uremic patients One of these—a patient with glomerulonephritis—showed a rise in properdin as his uremia subsided

Considerable data from animal experiments indicate that immune responses may be altered by administration of various tissues prior to transplantation of the same or related tissues Blood elements have been extensively studied in this regard[8, 14, 15] Because of therapeutic implications in

When an animal was desired with skin in the late growing phase (late anagen) the animal was plucked and used 12 days later (if the above changes had been noted). For telogen skin animals were plucked and, if satisfactory were used 25 days later. For skin in the early growing phase (early anagen) hair was plucked and following the usual changes was replucked 25 days later (while in telogen). The skin was used 4 days after replucking that is 29 days after the original plucking. Any animal in which the skin phase could not be identified accurately was discarded for the purpose of the experiment. Before grafting all remaining hair on the dorsum was removed with a commercial depilatory.*

A single donor animal was used to donate skin grafts to several recipient animals. In the grafting technique used the skin graft had the panniculus carnosus removed and the recipient bed had the panniculus carnosus and its associated blood vessels intact (modified from Edgerton).[11]

Donor skin was taken in each of the 3 phases and grafted to individual recipient beds in each of these phases. Control experiments were also done. These were autograft experiments (skin grafted from 1 animal to the same animal) to demonstrate the dependability of the method of grafting and isograft experiments (skin grafted from 1 individual of an inbred strain to another of the same strain) to demonstrate the genetic uniformity of the animals.

Each homograft experiment initially involved grafting skin from 1 Balb/c mouse to 4 $C_{57}$Bl/6 mice and from 1 $C_{57}$-Bl/6 mouse to 4 Balb/c mice. Whenever experimental results were in doubt (due to lack of sufficient animals surviving) or where a check on the results was desired additional grafting experiments were done.

The donor animal was anesthetized by intraperitoneal Nembutal** (0.85 mg/10 gm body weight). The operative area was prepared with a pHisohex*** and Zephiran**** scrub. Several donor sites (1.5 cm) were marked on the animal's dorsal skin using a template and methylene blue. Skin outside the marked area was then cut into with a small curved scissors. The scissors were used points up to create a cleavage plane between the panniculus carnosus and the fatty layer of the skin. The graft was cut along 3 sides and laid back. Any remaining panniculus was very carefully pulled away from the graft. Locke's solution was used to keep the tissues moist. The skin graft was put back in place while the recipient animal was prepared. When ready the fourth side was cut, the skin graft transferred to the recipient bed and the donor animal sacrificed.

The recipient animal was prepared in the same way with an area 1.3 cm square being marked on the dorsum using a template and methylene blue. The skin was then tented along 1 side of this area and a slight shallow cut made using small curved scissors. With traction on the cut skin edge in a direction opposite to the cutting of the scissors a narrow strip of skin was cut from the underlying panniculus carnosus around the 4 sides of the recipient site. The underlying panniculus carnosus and its associated blood vessels were kept moist with sterile Locke's solution. When the recipient site was

* Nair, Carter Products Inc., NYC, NY—The active ingredient is calcium thioglycolate.
** Nembutal, Pentobarbital, Abbott Laboratories, North Chicago, Ill.
*** pHisohex, Winthrop Stearns Inc., NYC, NY.
**** Zephiran Chloride, Winthrop Stearns Inc., NYC, NY.

work the possible importance of the skin cycles of the rodent has been overlooked. The changes seen in the skin during a skin cycle are very marked and could well introduce errors into experimental results.

Skin cycles are a regular synchronized waxing and waning of practically all of the morphological and biochemical components of the skin.[5, 9, 10, 11, 13] The skin cycle is divided into 3 phases. The first is a growing phase called *anagen* during which all hair growth in a given area takes place. This lasts about 18 days and is followed by a 2 day regressive phase called *catagen* and then by a 1 to 10 day resting phase called *telogen*.[10] The growing phase has been divided into substages on the basis of morphological changes in hair follicle development.[4, 5] Other factors such as the estrus cycle,[3] the environmental temperature,[14] the daily activity,[2] food ingestion,[1, 4] and minor trauma such as bites and scratches have been shown to cause appreciable changes in the day to day morphology and physiology of rodents' skin. In addition, it would appear that genetic differences between males and females of the same strain affect the survival of skin grafts.

When hair is pulled out in a given area (by plucking or by bites, scratches and cuts) the onset of a new cycle is hastened. This "induced" cycle is limited to the area plucked and involves all the skin components. Cutting the hair without pulling it out appears to have no effect on the skin cycle.[9, 17] If the hair in a given area is plucked in telogen, a new cycle begins almost immediately at the beginning of anagen. This makes it possible to predict and control the phase of the skin cycle.

During the first few naturally occurring cycles there is also some relationship between the age of the animal and the stage of the skin cycle on the animal's dorsum. However, this is probably not a reliable way of determining the stage of the skin cycle.[16]

## METHOD

C Bl/6 and Balb/c male mice from the Roscoe B. Jackson Memorial Laboratory were used to insure a constant difference between donor and recipient. The mice were isolated in individual cages in a room kept at 70±1°F. The skin cycle was controlled by inducing a new cycle on adult animals (over 90 days old) whose skin appeared to be in telogen. The dorsal hair was plucked by hand, and then several coats of an epilating wax* were applied to remove all remaining visible hairs.

To be absolutely certain of the phase of the skin, the plucked areas were inspected daily for the appearance of certain characteristic changes indicating the onset of anagen. These consist of a thickening of the skin by the 3rd day, darkening of the skin in pigmented animals by the 7th day, and eruption of the hair shaft by the 8th to 9th day. If the plucking is erroneously done in some phase other than telogen, these changes do not occur or they occur on a different time schedule.

Three phases of the skin cycle were chosen for investigation. The early growing phase (early anagen), the late growing phase (late anagen) and the resting phase (telogen). These phases were selected because each is markedly different from the others. In addition, early anagen was selected because grossly it is sometimes difficult to distinguish from telogen.

*Zip epilator, Jordeau, Inc., NYC, N.Y. This, when heated in a water bath to its melting point, was found to be satisfactory.

C Bl/6 mice were used The average survival time was 3 4 days The end point occurred on the 3rd day in 5 grafts and on the 4th day in 3 grafts

*G* Late anagen skin grafted to recipients in telogen 4 Balb/c and 4 C Bl/6 mice were used Two grafts were unsatisfactory and were discarded The remaining 6 grafts had an average survival time of 3 0 days All end points were on the 3rd day

*H* Late anagen skin grafted to recipients in early anagen 7 Balb/c and 8 C Bl/6 mice were used Eight grafts were unsatisfactory and were discarded The remaining 7 grafts had an average survival time of 3 3 days The endpoint occurred on the 3rd day in 5 grafts and on the 4th day in 2 grafts

*I* Late anagen skin grafted to recipients in late anagen 4 Balb/c and 5 C Bl/6 mice were used Two grafts were unsatisfactory and were discarded The remaining 7 grafts had an average survival time of 3 3 days The end point occurred on the 3rd day in 5 grafts and on the 4th day in 2 grafts

*Table 1 The Survival Time of Skin Homografts Measured in Days Showing Variations Due to the Phase of the Skin Cycle of the Graft and the Recipient Bed*

| | Recipient Bed | | |
|---|---|---|---|
| Skin Graft | Telogen | Early Anagen | Late Anagen |
| Telogen | 7 3 | 5 1 | 5 2 |
| Early Anagen | 4 2 | 3 6 | 3 4 |
| Late Anagen | 3 0 | 3 3 | 3 3 |

## Summary and Conclusion

These results indicate that telogen skin homografts survive as much as twice as long as anagen skin homografts and that they lasted longer than grafts in any other stage regardless of the phase of the cycle in the recipient bed The phase of the recipient bed does seem to exert some effect on the duration of the skin homograft survival This is most clearly demonstrated where telogen skin was grafted to anagen recipient beds These figures are thought to be significant because in any given group all the homografts displayed an endpoint reaction within a space of 48 hr at most In fact the endpoint in any 1 group occurred within a space of 24 hr in all but 3 mice of the 73 animals observed These results tend to confirm the work of Ballantyne and Converse in rats

It would seem that skin cycles exert an appreciable effect on the survival time of skin homografts in the mouse Skin cycles should probably be controlled in skin homograft experiments on the mouse the rat and the rabbit where the survival time of skin homografts is to be measured

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completely cleared the donor skin was freed and adjusted in place, the excess skin being trimmed so that the graft was tailored to fit the recipient area. No sutures, glue, or dressing were used. The animal was kept anesthetized for several hours to permit the graft to agglutinate in place.

The endpoint consisted of observing a subgraft hemorrhagic reaction which is visible to the naked eye as small ecchymotic areas. This usually occurs first in isolated areas, later spreading to include the entire graft. The skin overlying such hemorrhages becomes crusted and necrotic and eventually sloughs away. This hemorrhagic spot is remarkably abrupt in its onset and seems to bear a fairly constant relationship to the cessation of graft viability. It has been correlated by Edgerton[11] with the microscopic changes seen at the site of a homograft rejection reaction.

After grafting, the recipient animals were inspected at the end of each 24 hr interval to determine the condition of the graft. A few grafts displayed necrotic areas within 48 hr, possibly due to serious interference with the blood supply to the panniculus. The graft was considered unsatisfactory and was not included in the tabulation of results if it displayed extensive necrotic areas within 48 hr, if the animal died while still under anesthesia, or if the animal managed to remove the graft (always within the first 48 hr).

RESULTS

**Autograft Experiments.** Three Balb/c and 3 C57Bl/6 mice received autografts. Survival of the autograft was assumed if it lasted for more than 30 days and a growth of hair was noted on the graft. All 6 autografts survived.

**Isograft Experiments.** Twelve Balb/c and 7 C57Bl/6 mice were isograft recipients. Six grafts were unsatisfactory and were discarded. Survival of the isograft was assumed if it lasted for more than 30 days and a growth of hair was noted on the graft. The remaining 11 isografts survived.

**Homograft Experiments.** *A. Telogen skin grafted to recipients in telogen.* 9 Balb/c and 8 C57Bl/6 mice were used. Six grafts were unsatisfactory and were discarded. The remaining 11 grafts had an average survival time of 7.3 days. Endpoints occurred on the 6th day in 2 grafts, on the 7th day in 4 grafts, and on the 8th day in 5 grafts.

*B. Telogen skin grafted to recipients in early anagen.* 7 Balb/c and 8 C57Bl/6 mice were used. One graft was unsatisfactory and was discarded. The remaining 14 grafts had an average survival time of 5.1 days. Endpoints occurred on the 4th day in 1 graft, on the 5th day in 10 grafts, and on the 6th day in 3 grafts.

*C. Telogen skin grafted to recipients in late anagen.* 6 Balb/c and 8 C57Bl/6 mice were used. Five grafts were unsatisfactory and were discarded. The average survival time of the remaining 9 grafts was 5.2 days. Endpoints occurred on the 5th day in 7 grafts and on the 6th day in 2 grafts.

*D. Early anagen skin grafted to recipients in telogen.* 4 Balb/c and 4 C57Bl/6 mice were used. Two grafts were unsatisfactory and were discarded. The remaining 6 grafts had an average survival time of 4.2 days. The endpoint occurred on the 4th day in 5 grafts and on the 5th day in 1 graft.

*E. Early anagen skin grafted to recipients in early anagen.* 6 Balb/c and 4 C57Bl/6 mice were used. The average survival time was 3.6 days. The endpoint occurred on the 3rd day in 4 grafts and on the 4th day in 6 grafts.

*F. Early anagen skin grafted to recipients in late anagen.* 4 Balb/c and 4

Approximately six hundred 2 to 3 mm. square human skin explants from 31 patients were studied. These were taken as thin split thickness grafts in the operating or autopsy room. The skin was preserved from 1 to 70 days at 4° C. in 10 ml. of a nutrient media consisting of 10 per cent human placental cord serum, 90 per cent Simms' X7 balanced salt solution and 100 units of penicillin/ml.

Two tissue culture methods were utilized: the Maximow lying drop double cover slip and Carrel D5 flasks. The supporting clot for both methods consisted of 1 part chick plasma (Difco), 1 part 9 day chick embryo extract with Simms' X7, 1 part human placental cord serum and 100 units of penicillin/ml. The liquid nutrient consisted of 2 parts human placental cord serum, 1 part Simms' X7, 1 part 9 day chick embryo extract and 100 units penicillin/ml. The liquid nutrient was added to the supporting clot every 3 days beginning on the third day with the lying drop and on the first day with the Carrel D5 flasks.

Results indicated adequate growth of fibroblasts and epidermal elements, often however after a lag period of some days for the thicker skin explants. Ordinary operating room skin preps failed to insure the rigid sterility required for tissue culture. Contamination could be routinely prevented only if an antibiotic in proper concentration were added. Also certain skin preparation agents cause a chemical toxicity to sterile tissue cultures.

Viable cultures were obtained from living patients up to 21 days after preservation at 4°C. in the 10 per cent serum nutrient media. However, longer preservation times decreased the chances for a viable culture as indicated in the tabular data. Viable cultures were obtained from postmortem skin taken up to 10 hr. after death and preserved at 4°C. in the 10 per cent serum nutrient media up to 11 days.

Criteria of viability for preserved human skin grafts is a difficult subject but many workers feel that tissue culture methods offer the best qualitative

*Table 1. Tissue Culture Results of Human Skin Grafts*

| STORAGE TIME | NUMBER OF PATIENTS | GROWTH RESULTS |
|---|---|---|
| 1–3 days | 8 | 8 Good |
| 4–8 days | 4 | 2 Good, 1 None, 1 Contaminated |
| 9–15 days | 4 | 2 Good, 2 None |
| 16–28 days | 4 | 1 Good, 2 None, 1 Contaminated |
| 29–70 days | 5 | None |

*Table 2. Tissue Culture Results of Postmortem Human Skin Grafts*

| TIME AFTER DEATH | STORAGE TIME | GROWTH RESULTS |
|---|---|---|
| 2 hr. | 11 days | Good |
| 4 hr. | 8 days | Good |
| 6 hr. | 1 day | Good |
| 8 hr. | 5 days | Good |
| 9 hr. | 6 days | Good |
| 10 hr. | 1 day | Poor |

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## VIABILITY STUDIES OF HUMAN SKIN GRAFTS WITH TISSUE CULTURE METHODS*

ROBERT M MCCORMACK

Numerous human tissues preserved at low temperatures may be clinically useful as transplanted tissues without the exacting criteria of viability of the preserved cells However when skin is required as a transplanted tissue the transplanted cells must remain viable to be permanently clinically useful The permanent replacement of viable epidermis for a full thickness skin loss of large size can be obtained only from a viable skin autograft (fresh or preserved) The clinical necessity for viable human skin autografts points to the need for further laboratory information on the criteria and conditions to insure this viability As Allgower and Blocker[1] point out tissue culture studies are very exacting tests for survival studies of stored skin Tissue culture is generally considered a more stringent test than reimplantation on the donor or performing a heterograft to a laboratory animal

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With the technical assistance of Miss Phyllis Peters

# THE DIFFERENTIAL STAINING OF THE PAROTID GLAND AS A PRELIMINARY TO PAROTID SURGERY*

HERBERT J. FORREST

A project was set up with the specific aim of finding a method of staining the parotid gland in the live animal without staining the surrounding tissue or facial nerve. The anatomy of the parotid gland, Stensen's duct, and facial nerve of the dog was studied. This was done by dissecting dog cadavers. It was found that the relationship between the facial nerve and the parotid gland was similar to man's except in the dog the branches of the facial nerve were relatively larger and the parotid gland lay almost entirely superficial to the nerve. Usually a small projection of the parotid gland extended down between the 2 main branches of the facial nerve. The parotid duct was found to be small and fragile and sometimes difficult to identify.

## METHOD

The experimental work was done using live dogs under anesthesia. Aseptic operative technique was used. In each case Stensen's duct was exposed as it crossed the masseter muscle. A small needle was inserted into the duct and ½ to 3 cc. of dye was injected into the gland. Only relatively nontoxic dyes were tried, e.g., indigo carmine, Evans blue and methylene blue. Parotidectomies were done at varying intervals following the injection. Twenty-four operative procedures have been done to date.

## RESULTS

It was found that in the live dog the parotid gland could be stained precisely and completely without staining the surrounding tissue or facial nerve. With the gland exposed it was seen to distend and take on the color of the dye. The gland capsule was an effective barrier and contained the dye. The facial nerve remained white and vivid against the color of the stained gland.

Evans blue in .15 per cent solution injected into the parotid duct system stained the gland very well. However, it had the disadvantage of being picked up by the lymphatic system. If immediate parotidectomy was not done, the surrounding face and neck subcutaneous tissue would take on a light blue color.

Indigo carmine in .8 per cent solution stained the parotid gland adequately but was less bright than Evans blue or methylene blue. Methylene blue in 1 per cent solution was studied most because it seemed to do the best job of staining the gland. Microscopic study of methylene blue stained parotid tissue was done using frozen and paraffin imbedded sections. It was believed that the methylene blue entered *only* the ramifications of the parotid duct system. No staining or damage to the gland cells could be seen.

## DISCUSSION

It was found that there was no advantage in waiting for the gland to take up the stain. Immediate parotidectomy could be done without any danger of dye leakage even if the gland tissue was invaded. In the dog the parotid

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evidence of intact cellular function under the most rigid controls. Many criteria have been utilized: for example, the gross and microscopic appearance of human skin grafts on the chorioallantoic membrane of chick embryos (Tenery and McDowell[2]) and on autotransplantation to granulating surfaces (Allgower and Blocker,[1] Georgiade *et al.*,[3] and Brown *et al.*[4]), biochemical criteria such as oxygen uptake utilized by Skoog[5] and Georgiade *et al.*,[3] and enzymatic activity by Chamness *et al.*[6] Heterografts from human to the mouse have been used for large scale testing (Brown *et al.*[7]) by gross appearance.

Relatively standard tissue culture techniques with available commercial media can make such techniques practical for the evaluation of viability of preserved human skin grafts. Such a laboratory check should be used on preserved postmortem homografts, as there is a considerable clinical difference between use as a viable temporary homograft that takes and a nonviable dressing material that fails to take, macerates within a few days and may postpone the time when permanent healing with autografts can be accomplished.

## SUMMARY

1. Tissue culture of human skin grafts, particularly after storage, is an exacting qualitative test of viability.

2. Viable skin tissue cultures were obtained up to 3 wks. after storage in a 10 per cent serum nutrient media at 4°C.

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# THE DIFFERENTIAL STAINING OF THE PAROTID GLAND AS A PRELIMINARY TO PAROTID SURGERY*

HERBERT J FORREST

A project was set up with the specific aim of finding a method of staining the parotid gland in the live animal without staining the surrounding tissue or facial nerve The anatomy of the parotid gland Stensen s duct and facial nerve of the dog was studied This was done by dissecting dog cadavers It was found that the relationship between the facial nerve and the parotid gland was similar to man s except in the dog the branches of the facial nerve were relatively larger and the parotid gland lay almost entirely superficial to the nerve Usually a small projection of the parotid gland extended down between the 2 main branches of the facial nerve The parotid duct was found to be small and fragile and sometimes difficult to identify

## METHOD

The experimental work was done using live dogs under anesthesia Aseptic operative technique was used In each case Stensen s duct was exposed as it crossed the masseter muscle A small needle was inserted into the duct and ½ to 3 cc of dye was injected into the gland Only relatively nontoxic dyes were tried e g indigo carmine Evans blue and methylene blue Parotidectomies were done at varying intervals following the injection Twenty four operative procedures have been done to date

## RESULTS

It was found that in the live dog the parotid gland could be stained precisely and completely without staining the surrounding tissue or facial nerve With the gland exposed it was seen to distend and take on the color of the dye The gland capsule was an effective barrier and contained the dye The facial nerve remained white and vivid against the color of the stained gland

Evans blue in 15 per cent solution injected into the parotid duct system stained the gland very well However it had the disadvantage of being picked up by the lymphatic system If immediate parotidectomy was not done the surrounding face and neck subcutaneous tissue would take on a light blue color

Indigo carmine in 8 per cent solution stained the parotid gland adequately but was less bright than Evans blue or methylene blue Methylene blue in 1 per cent solution was studied most because it seemed to do the best job of staining the gland Microscopic study of methylene blue stained parotid tissue was done using frozen and paraffin imbedded sections It was believed that the methylene blue entered *only* the ramifications of the parotid duct system No staining or damage to the gland cells could be seen

## DISCUSSION

It was found that there was no advantage in waiting for the gland to take up the stain Immediate parotidectomy could be done without any danger of dye leakage even if the gland tissue was invaded In the dog the parotid

* From the Department of Plastic Surgery University of Kansas Medical Center Kansas City Kansas

gland appeared to blend with the surrounding areolar tissue After being stained it was outlined precisely and could be dissected readily Finally separation of the facial nerve from parotid tissue was made much easier

The only complication noted was some edema of the adjacent subcutaneous tissue if the stained gland was left in place This was found if the face was reopened 1 to 4 days after the dye injection The surgical wound healed well in all cases after the parotidectomy Also the surgical wound healed well if the stained parotid gland was left *in situ*

Preliminary clinical trial has been done This has shown that the human parotid can be stained with an intra-ductal injected dye Results of clinical use of this method of staining the parotid gland will be detailed in a later paper

SUMMARY

The parotid gland of the dog can be stained by injecting 1 5 to 3 cc of an appropriate dye into Stensen s duct No other tissue outside the gland is stained Parotidectomy and separation of the facial nerve from the gland is greatly facilitated by preliminary staining of the parotid gland It is hoped this method of dyeing the parotid gland can be utilized to simplify human parotid surgery

*Urology*

# A STUDY OF THE PATHOLOGIC ANATOMY OF THE PROXIMAL TUBULES OF THE MOUSE PRODUCED BY VARIOUS NEPHROTOXINS*

C. BARBER MUELLER AND A. D. MASON, JR.

The pathologic anatomy of renal tubular lesions is less well understood than the pathologic physiology of acute renal failure, which has been of great interest in recent years. The clinical course of patients suffering from acute renal failure following the ingestion of nephrotoxins is somewhat different and seemingly less fulminant than that of acute renal failure which follows crushing or wounding, and although extrarenal factors may well be responsible for this apparent dissimilarity, it is possible that differences in tubular pathology may be the cause. In a previous communication [1] electron micrographs of normal dog kidneys and of dogs subjected to repeated transfusions of human blood were presented. The refined techniques of electron microscopy which are now currently in use have already outdated this previous study, but some information of a comparative nature may be drawn between this present work and the prior report.

This study is a preliminary description of specific changes in the proximal tubule cells of mouse kidneys following the administration of mercuric chloride, uranyl nitrate and potassium dichromate.

## METHOD

White female mice weighing 25 to 32 gm and between 100 and 120 days old were used. In the mercury bichloride experiments 1.0 mg of $HgCl_2$ in 1.0 ml of water was injected subcutaneously and animals were sacrificed at 2, 4 and 6 hr. We had previously determined that 1.0 mg $HgCl_2$ killed mice in 36 to 72 hr. No animal survived the injection of 1.5 mg more than 3 hr and 2 mice given 0.25 mg survived for 3 wks. When animals were sacrificed 3 hr after the administration of 1.0 mg $HgCl_2$ the kidneys were usually pink, while by 4 to 6 hr they had become pale though not particularly swollen. The mice did not appear particularly ill by 6 hours.

In another group of mice 1.0 mg of uranyl nitrate was administered subcutaneously, and in a third group 2.5 mg of potassium dichromate was injected intraperitoneally. These animals were handled in a fashion similar to those in the mercuric chloride group.

Portions of the kidneys were fixed in 10 per cent formalin, while specimens for electron microscopy were cut into 1 mm cubes and dropped into Dalton's osmic acid fixative. After dehydration in alcohol the specimens were embedded in methacrylate, sectioned with a glass knife and examined in an RCA EMU electron microscope.

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* From the Department of Surgery, Washington University, St. Louis, Mo. Supported by U.S.P.H.S. Grant A-96 (C4).

RESULTS

Routine formalin fixed sections embedded in paraffin and stained with hematoxylin and eosin, do not show any necrosis or sloughing of the tubules at this time

After mercuric chloride and uranyl nitrate injections examination of *proximal distal and collecting tubules with the electron microscope confirmed* the light microscope impression that there was no gross destruction of cellular integrity However all cells showed a progressive dissolution of the mitochondria There seemed to be fewer deranged mitochondria in the pink kidneys of the mice sacrificed at 3 hr than of the pale kidneys seen at 6 hr but a statistical evaluation is not possible with such small samples All cells particularly in those of the proximal convoluted tubules contained all stages of mitochondrial dissolution from normal intact mitochondria to pale washed out disrupted ghosts The pale 6 hr kidneys contained mostly ghosts the pink 3 hr kidneys mostly well formed mitochondria but in both groups all stages were recognizable Basement membranes cell borders brush borders and the background cytoplasm of the cells appeared intact

The normal mouse mitochondrion has been well described and pictures of it are not presented here There is a double membrane around the periphery and transverse cristae which are also comprised of a double membrane Each single membrane is approximately 50 A° in thickness and the space between them 70 A° [2, 3] The mitochondrial material itself is much more electron dense than the background cytoplasm of the cell and is generally homogenous throughout the mitochondrion except for a dense spherical body 300 to 500 A°, which is seen in most mitochondria

Figure 1 is a low power view of a proximal tubule cell of a mouse poisoned with mercuric chloride In the lower portion is the basement membrane at the top are under portions of the brush border In the central portion are three fairly normal mitochondria (a) Disruption of the peripheral membranes can be seen at (b) while cristae remain intact Vacuolization of the



Fig 1 Mitochondrial dissolution 3 hr following mercuric chloride (For explanation of figures see text)



Fig. 2 Mitochondrial dissolution following mercuric chloride

*mitochondrion with progressive loss of cristae is seen at (c) and the hollow* disrupted completely vacuolated mitochondrial ghost at (d)

Figure 2 shows several phases of the early peripheral membrane disruption (b) and vacuolization (c) This kind of degenerative changes seems to be the most common Poisoning with uranyl nitrate gives lesions similar in all respects to those seen with mercuric chloride

Figure 3 shows at high magnification the loss of peripheral membrane (b) and disruption (c) of the internal structure



Fig. 3 Mitochondrial dissolution following mercuric chloride

RESULTS

Routine formalin fixed sections, embedded in paraffin and stained with hematoxylin and eosin, do not show any necrosis or sloughing of the tubules at this time.

After mercuric chloride and uranyl nitrate injections, examination of *proximal, distal and collecting tubules with the electron microscope con*firmed the light microscope impression that there was no gross destruction of cellular integrity. However, all cells showed a progressive dissolution of the mitochondria. There seemed to be fewer deranged mitochondria in the pink kidneys of the mice sacrificed at 3 hr than of the pale kidneys seen at 6 hr but a statistical evaluation is not possible with such small samples. All cells, particularly in those of the proximal convoluted tubules, contained all stages of mitochondrial dissolution from normal intact mitochondria to pale washed-out disrupted ghosts. The pale (6 hr) kidneys contained mostly ghosts, the pink (3 hr) kidneys mostly well formed mitochondria, but in both groups all stages were recognizable. Basement membranes, cell borders, brush borders and the background cytoplasm of the cells appeared intact.

The normal mouse mitochondrion has been well described and pictures of it are not presented here. There is a double membrane around the periphery and transverse cristae which are also comprised of a double membrane. Each single membrane is approximately 50 A° in thickness and the space between them 70 A°.[2,3] The mitochondrial material itself is much more electron dense than the background cytoplasm of the cell and is generally homogeneous throughout the mitochondrion except for a dense spherical body 300 to 500 A°, which is seen in most mitochondria.

Figure 1 is a low power view of a proximal tubule cell of a mouse poisoned with mercuric chloride. In the lower portion is the basement membrane; at the top are under portions of the brush border. In the central portion are three fairly normal mitochondria (a). Disruption of the peripheral membranes can be seen at (b) while cristae remain intact. Vacuolization of the



Fig 1 Mitochondrial dissolution 3 hr following mercuric chloride (For explanation of figures see text)




Fig 2 Mitochondrial dissolution following mercuric chloride

mitochondrion with progressive loss of cristae is seen at (c) and the hollow disrupted completely vacuolated mitochondrial ghost at (d)

Figure 2 shows several phases of the early peripheral membrane disruption (b) and vacuolization (c) This kind of degenerative changes seems to be the most common Poisoning with uranyl nitrate gives lesions similar in all respects to those seen with mercuric chloride

Figure 3 shows at high magnification the loss of peripheral membrane (b) and disruption (c) of the internal structure




Fig 3 Mitochondrial dissolution following mercuric chloride

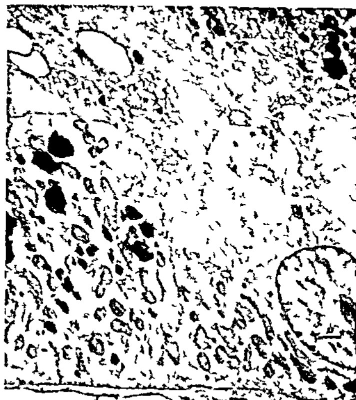


Fig. 4. Cellular changes following administration of potassium dichromate.

Figure 4 shows the pale cytoplasm, shrunken, condensed mitochondria and crisp fixed cell membranes which are present following the administration of potassium dichromate.

DISCUSSION

Simonds and Hepler[4] have presented evidence that mercuric chloride and uranyl nitrate damage the distal portion of the proximal tubule while potassium dichromate has a predilection for its more proximal portions. The work presented here indicates that uranyl nitrate and mercuric chloride attack a similar portion of the tubule and produce similar effects within the cell, while potassium dichromate creates an entirely different lesion both as to locus in the tubule and type of degeneration in the cell. All 3 of these nephrotoxins produce renal tubule disease which in its pathologic anatomy appears to be different from that caused by the infusions of mismatched blood into dogs.[1]

CONCLUSIONS

Mercuric chloride and uranyl nitrate cause renal tubular necrosis that starts with dissolution and vacuolization of the mitochondrion.

Potassium dichromate causes *renal tubular necrosis that commences* with mitochondrial coalescence, a "washed out" appearance in the background cytoplasm, and alterations of the cell membranes.

Electron microscope pictures visualizing these changes are presented.

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# MECHANISMS OF HYDRONEPHROSIS THE ROUTE OF BACKFLOW*

LESTER PERSKY FREDERICK J BONTE AND CHARLES A HUBAY

The initial stimulatory studies by Hinman[1] concerning the nature of hydronephrosis demonstrated that pelvic contents in cases of obstruction gain access to the blood stream A variety of substances[2, 3] varying from dyes to bacteria have been utilized since then in the pursuit of the exact route of this backflow The development of radioactive isotopes and exact counting techniques suggested that the use of these substances would afford a more accurate technique for a study of hydronephrosis and a more definitive tracing of the reflux In previous communications[4-5] we showed that particle size did not mitigate against backflow, that the backflow occurred rapidly in the acutely obstructed kidney and by means of radioautographs we demonstrated that this access to the vascular tree appeared to be a combination of pyelotubular and pyelointerstitial routes

The recent work of Goodwin[6] coupled with contributions from the foreign literature[7, 8] focused our attention upon the renal lymphatics This study was undertaken in order to prove or disprove the role of these channels in hydronephrosis

## METHOD

All experiments were carried out on healthy mongrel dogs weighing from 10 to 14 kg Nembutal anesthesia was employed routinely After exposing the kidney complete obstruction was created by ligating the ureter close to the ureteropelvic junction about a special 3 way needle leading to a mercury manometer and through which radioactive isotopes could be instilled into the pelvis without modifying intrapelvic pressures This was done at regular 5 min intervals throughout the course of each experiment to permit and insure complete mixing of urine and the radioactive substance Two hun

From the Department of Surgery Urological Service and the Department of Radiology Western Reserve University and the University Hospitals Cleveland Ohio Supported in part by the Beaumont Fund Cleveland Ohio and conducted in part with facilities made available through contract number W 31 109 ENG 78 between the United States Atomic Energy Commission and Western Reserve University

dred and fifty microcuries of iodinated albumin ($I^{131}$) (molecular weight approximately 60 000) were used for each animal This substance was utilized because of the large particle size which eliminated the possibility of simple diffusion and absorption

The animals were divided into 3 experimental groups In Group 1 the kidney was completely mobilized from all perirenal fat and the hilar vessels completely stripped of their surrounding fat, areolar tissue, nerves and presumably lymphatics After obstructing the ureter and after the intermingling of the pelvic contents with iodinated albumin as previously described blood samples were drawn at 5 min intervals for 1 hr

In Group 2 the thoracic duct was cannulated with a small polyethylene catheter close to its entrance at the junction of the subclavian and jugular veins in the neck The ureter was again ligated about the 3 way needle disturbing the kidney as little as possible and blood and lymph collected at 5 min intervals for 1 hr In Group 3 a similar experiment was carried out except that the kidney was once more completely mobilized and the renal vessels stripped Four animals were employed in Groups 1 and 2 and 2 animals in Group 3

## RESULTS

Consistent results were obtained in all animals within each group In Group 1 where the kidney and vessels were stripped radioactivity in the blood stayed at low levels but gradually rose slightly at the end of the experimental period of observation A typical animal of this group is seen in Figure 1 In Group 2 in which the kidney was undisturbed and the thoracic duct cannulated the radioactivity in the blood stream was of the same order of magnitude as was noted in Group 1 but the counts obtained from the thoracic duct lymph were elevated to a much greater degree (Fig 2) In Group 3 (Fig 3) with the kidney stripped again and a cannula in the thoracic duct we once again have the same general level of counts obtained in the peripheral blood but no or little radioactivity detected in the lymph The scale of these figures although not exactly the same has been modified to permit representation of the counts and does not distort the findings If exactly parallel scales had been used the blood levels in Groups 1 and 3 would have been barely discernible

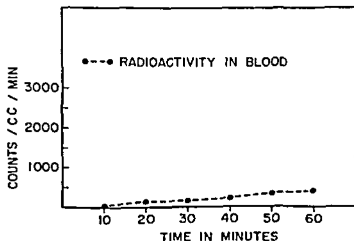



Fig 1 Representative curve of radioactivity detected in the blood stream following the installation of iodinated albumin in the obstructed renal pelvis of the completely mobilized kidney

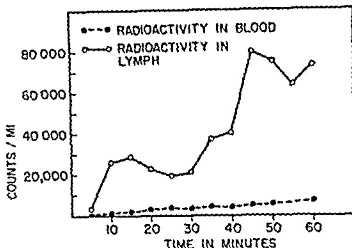



Fig 2 Representative curves of radioactivity detected in the blood and thoracic duct lymph following the installation of iodinated albumin in the obstructed pelvis of an undisturbed kidney

DISCUSSION

These studies corroborate the contention of Goodwin and others that the renal lymphatics play an important role in the mechanism of hydronephrosis The counts in the thoracic duct lymph exceed by far any of the previously observed levels in our initial studies (Fig 4) wherein radioactivity was noted in the peripheral blood following the installation of iodinated albumin ionic $I^{131}$ and colloidal gold The level of radioactivity in the blood detected following the use of colloidal gold is misleading due to the rapidity of the reticuloendothelial system in clearing it from the blood stream It is presumed that in the present experiments in which the kidney was completely mobilized and the vessels denuded that the lymphatics were divided completely The ultimate appearance of the iodinated albumin in the blood stream in all groups may represent reflux into capillaries secondary to pyelo interstitial backflow as suggested by Fuchs[9] and Narath[10] or may be a reflection of a few lymphatics which were undivided and had alternative entrances into the blood stream

The pattern of the curves of radioactivity found for the thoracic duct lymph in Group 2 is analogous to the curves found previously in the blood when the duct has not been cannulated and the kidney not disturbed The peak corresponds to the period after the fall in pelvic pressure and which

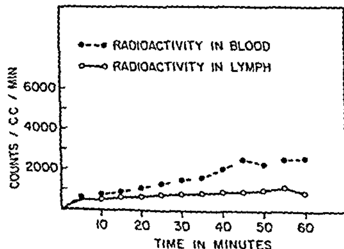



Fig 3 Radioactivity in the blood and lymph after completely mobilizing the kidney

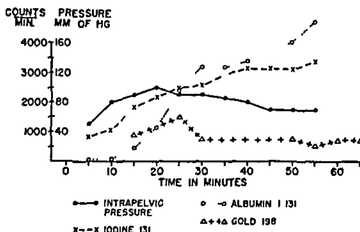



Fig 4 Representative curves for the backflow obtained utilizing iodinated albumin $I^{131}$ and colloidal gold

usually heralds the onset of backflow It is probably coincidental to the escape of pelvic fluid via the tubules or interstitial spaces into the lymphatics[4, 6] It is hoped ultimately to reduplicate these experiments in more chronic states of obstruction and in situations where the perirenal lymphatics have been modified by inflammatory or sclerosing agents If no backflow is obtained under these conditions or under conditions of auto or homotransplantation, then the evidence will be almost complete for the thesis to which we now subscribe, namely that the renal lymphatics are the main route of backflow in cases of obstruction

SUMMARY

In a series of experiments utilizing iodinated albumin in the obstructed kidney of the dog by cannulation of the thoracic duct the renal lymphatics were demonstrated to be the main route of backflow When the lymphatics were divided, radioactivity in the lymph fell to low levels further supporting this concept

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# HYDRODYNAMICS OF THE HUMAN URETER AND RENAL PELVIS*

WILLIAM H RATTNER SIDNEY LINK AND JOHN J MURPHY

The problems of hydronephrosis hydroureter, ureterovesical reflux, etc, confront the urologist frequently The solution to these problems on a physiologic basis requires an understanding of the normal hydrodynamics in the upper urinary tract While considerable knowledge has been gained from recording bladder pressures and activity relatively little is known about the basic pressure phenomena of the renal pelvis and ureter This study was done to record the basal pressure and peristaltic activity of the intact human ureter and renal pelvis

## METHOD

Seventeen adult females with normally functioning urinary tracts were studied Women were chosen because of the ease with which urethrocystoscopy could be carried out and a ureteral catheter could be held securely The subjects all had a recent normal intravenous urogram, and a normal blood urea nitrogen determination A #4 F ureteral catheter was passed to the renal pelvis and a #20 F catheter inserted into the bladder The catheters were connected to Statham P23B electrical transducers The resulting current was amplified and recorded by means of a Sanborn Poly Viso Recorder equipped with strain gauge amplifiers A simultaneous chest pneumograph was taken After a suitable period of recording from the renal pelvis the ureteral catheter was withdrawn 7 to 9 cm so that its tip was located in the midureter and another tracing made The catheter was then withdrawn an additional 8 to 10 cm to the lower third of the ureter, and the recording continued The location of the tip of the ureteral catheter was established by a plain film of the abdomen on several occasions Patency of the recording system was assured by noting pressure changes caused by lifting the head straining and deep breathing The patients received no premedication or anesthesia The volume of urine excreted during the test was measured and its pH recorded

## RESULTS

**Pressure and Amplitudes** Definite periodic pressure changes were observed on records from the renal pelvis mid and lower thirds of the ureter in all cases The absolute pressure values are recorded in Figure 1 All values (basal pressure and amplitude) are measured as centimeters of water pressure The basal pressure in the renal pelvis varied from 6 to 25 cm with an average of 14 7 The average amplitude of waves in the renal pelvis was 4 1 cm Similar amplitudes were noted in the mid ureter although the basal pressure was slightly higher (17 cm) The basal pressure in the lower third of the ureter was 14 3 cm but the amplitude of the periodic pressure changes was significantly higher They averaged 8 9 cm of water pressure

**Pattern of Waves** At least 4 distinct wave patterns were encountered These were the monophasic diphasic, triphasic and a slow undulating wave

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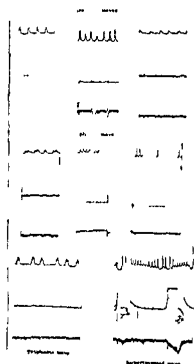


Fig. 1. The appearance of the phasic waves in the renal pelvis and ureter as recorded through an open lumened catheter. Simultaneous ureteral (top), bladder (middle), and pneumographic (lower) pressure recordings. Amplitude: One mm. equal to 1 cm. of water pressure except where indicated in the lower right record. Speed: 0.25 mm./sec. (3 large squares/min.) except in the upper and lower left records, where 6 large squares represent 1 min.

upon which other types of waves were superimposed (Fig. 1). The latter occurred only once in the study in association with a voiding contraction of the bladder. The other wave patterns were observed in each of the 3 areas studied. Different types of waves occurred at the same level, and in the same patient. While the waves usually had a definite rhythm, it was not unusual to find isolated bursts of 2 (couplets) or 3 (triplets) (Fig. 2). One patient was given Lorine (tricyclamol chloride) in a dose sufficient to cause xerostomia, but this drug did not in any way influence the ureteral activity in this patient. One patient with megaloureter was studied and found to have renal and ureteral pressure recordings similar to those from the normal ureter and renal pelvis. The urine volume and pH did not appear to have any correlation with the peristaltic activity in this study.

## DISCUSSION

Several methods have been used in the study of renal pelvic and ureteral motor physiology.[1] These include direct observation of the isolated and intact ureter, cystoscopic evaluation of the size and force of ureteral peristalsis,[2, 3] direct observation of the exteriorized trigone,[4] measurement of the action potentials of isolated and intact ureteral segments,[5, 6, 7, 8] cinefluorography,[9] and pressure studies using the hydrophorometer or one of its modifications.[10, 11, 12, 13, 14] None of these techniques recorded absolute pressure values, and most of them required subjective interpretation of the measurements. *In addition, the introduction of a balloon or large cannula produces* an abnormal pattern of peristalsis in any hollow viscus. The authors feel that the use of a fine ureteral catheter connected to a sensitive external recording device produces the least possible constant artifact in a study of this kind. A similar technique has been used recently in cardiac and gastrointestinal pressure studies.[15, 16] The basal pressures in all 3 segments studied were relatively constant, but it is interesting to note that the lower third of the ureter

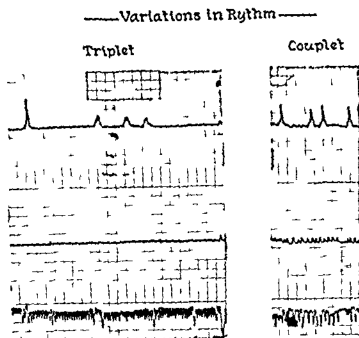



Fig 2 Simultaneous ureteral (top) bladder (middle) and pneumographic (bottom) tracings showing 3 waves occurring together ("triplet") and a two wave sequence ("couplet") Amplitude 1 mm represents 1 cm of water pressure Time On the left 0.25 mm /sec (3 large squares/min) On the right 0.5 mm /sec (6 large squares/min)

had the most vigorous peristaltic activity This may explain why reflux is so common after reimplantation of the ureter If in addition to ablating the natural valve at the ureterovesical junction one also removes the most active peristaltic segment reflux would seem almost inevitable The isolated observation on one patient who was given an anticholinergic drug would tend to lend support to the earlier observations of Lapides although more determinations are necessary in this field This technique should be of value in studying the pressure relationships in hydronephrosis and other malfunctions of the renal pelvis as well as the problems of ureteral reflux, megaloureter the effect of transplantation on the ureter and the influence of drugs on the motor activity of the urinary tract

SUMMARY

1 The basal pressure and peristaltic activity of the renal pelvis and ureter were studied in 17 normal adult females by means of an indwelling ureteral catheter connected to an external electrical transducer and strain gauge amplifier

2 Four distinct wave patterns were found These were the monophasic diphasic triphasic and slow undulating wave upon which other waves were superimposed

3 The average amplitude of the peristaltic waves was highest in the lower third of the ureter

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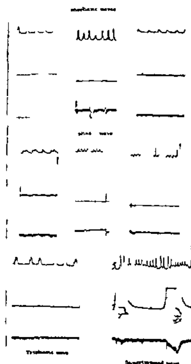


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of the fluid bathing the ureteral segment was correlated with the action potentials recorded from the electrodes positioned in the ureter contained in the peritoneal recess The lag of temperature change in the ureteral wall compared to that of the fluid about it, was measured in 2 experiments by using a needle thermocouple placed in the ureteral muscle The effects of drugs upon ureteral activity were studied by placing drug solutions in peritoneal recesses by intravenous administration, and by injection into the ureteral wall Conduction of waves in ureters having healed anastomoses also was investigated [2]

## RESULTS

**Normal Peristaltic Action Potentials** The action potential of a single peristaltic wave consisted of a spike followed by partial repolarization a plateau and completion of repolarization When the peristaltic wave originated in the ureter away from an electrode its action potential possessed an approach artefact (Table 1 1) When a wave arose at an electrode, no approach artefact was seen That a peristaltic wave arose at a given electrode was also evidenced by its simultaneous appearance at equidistant electrodes above and below the electrode of origin In most such waves, a slow rise of negativity (prepotential) was recorded from the electrode at the site of wave origin before the onset of the action potential spike (Table 1 4)

**Post peristaltic Interval of Reduced Wave Velocity** If 1 wave follows

*Table 1 A Summary of Action Potential Shapes*

| | Tissue | Position of the Electrode | Temperature | Wave Interval | Recorded Action Potential |
|---|---|---|---|---|---|
| 1 | Ureter | Intraluminal away from site of wave origin | 37° C | 5 plus seconds | |
| 2 | Ureter | As in (1) | 37° C | 1 second | |
| 3 | Ureter | As in (1) | 28° C | 5 plus seconds | |
| 4 | Ureter | Intraluminal at site of wave origin | 37° C | 5 plus seconds | |
| 5 | Ureter | In lumen of a healed anastomosis | 37° C | 5 plus seconds | |
| 6 | Ureter | Intraluminal near healed anastomosis | 37° C | 5 plus seconds | |
| 7 | Cardiac | Intracellular pacemaker cells | 37° C | 0 6 seconds | |
| 8 | Ureter | Postulated true shape of the action potential at 37° C after a wave interval of 5 or more seconds | | | 2.0 MV; 1.0 SEC. |

Canine ureteral action potentials are shown in lines 1 to 6 inclusive Line 7 shows the presence of a prepotential recorded intracellularly from pacemaker cells of cardiac muscle Line 8 outlines the postulated true shape of the ureteral action potential Whether or not the prepotential persists in an action potential conducted beyond its site of origin is as yet unknown

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# VARIATIONS IN THE SHAPE OF PERISTALTIC ACTION POTENTIALS OF THE CANINE URETER*

HARVEY R BUTCHER JR AND WILLIAM SLEATOR JR

Despite many investigations of ureteral peristalsis the mechanism of its propagation along the ureter remains poorly understood Consequently, a study of canine ureteral action potentials and the factors affecting their shape was undertaken to define accurately the electrical properties of the peristaltic conduction mechanism in an attempt to learn more concerning the transmission of the contraction wave through ureteral smooth muscle

## METHODS

Adult mongrel dogs of both sexes were anesthetized by intravenous injection of sodium pentobarbital (0 03 gm /kg) A cystostomy permitted easy intraureteral placement of an electrode assembly through the ureterovesical orifice A multi-channeled modified Offner dynograph recorded the action potentials of peristaltic waves from the intraluminal electrodes of fine silver wire (0 01″ in diameter) [1, 2] Waves were stimulated by distension of a small rubber balloon inserted into the upper ureter

The effect of different temperatures upon ureteral conduction was studied by placing mammalian Ringer s solutions of varying temperatures into a peritoneal recess constructed about a segment of the ureter Temperature

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of the fluid bathing the ureteral segment was correlated with the action potentials recorded from the electrodes positioned in the ureter contained in the peritoneal recess The lag of temperature change in the ureteral wall compared to that of the fluid about it was measured in 2 experiments by using a needle thermocouple placed in the ureteral muscle The effects of drugs upon ureteral activity were studied by placing drug solutions in peritoneal recesses by intravenous administration, and by injection into the ureteral wall Conduction of waves in ureters having healed anastomoses also was investigated[2]

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| 3 | Ureter | As in (1) | 28° C | 5 plus seconds | |
| 4 | Ureter | Intraluminal at site of wave origin | 37° C | 5 plus seconds | |
| 5 | Ureter | In lumen of a healed anastomosis | 37° C | 5 plus seconds | |
| 6 | Ureter | Intraluminal near healed anastomosis | 37° C | 5 plus seconds | |
| 7 | Cardiac | Intracellular pacemaker cells | 37° C | 0 6 seconds | |
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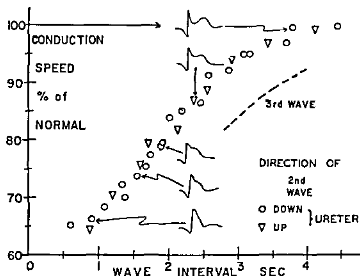



Fig 1 The Effect of Wave Interval The reduction in velocity and the change in action potential repolarization accompanying interperistaltic wave intervals of 3 sec or less is shown These changes were unaffected by the direction of propagation of the second wave along the ureter The dotted line labelled third wave shows the further reduction in velocity that occurs in a third wave stimulated at intervals of 4 sec or less after the second one

another within 3 to 6 sec, the speed of the second wave is less than the first[1] During the interval of reduced velocity the voltage time contour of the action potential of the peristaltic wave is altered (Fig 1) In the earlier part of this period that is just after passage of the distention refractory period (0 5 to 0 6 sec after the peristaltic wave) there is a reduction in the total duration of the action potential a broadening of the negative peak phase with little change in voltage and no separation of repolarization into 2 phases by a plateau Previous investigation[1] has correlated the degree of these action potential and velocity changes with a corresponding lessening of the strength of ureteral contraction

**The Effect of Different Temperatures Upon Peristaltic Action Potentials** Lowering of the temperature of an ureteral segment with cold mammalian Ringer s solution decreased the velocity of conduction lowered the voltage of the plateau of the action potential and transformed it into oscillations (Fig 2) When the temperature of the solution immediately adjacent to the segment of ureter reached 22° to 24 C conduction through it ceased At temperatures just above that required to produce block the velocity was the least and the plateau frequently not observed

Elevation of the temperature of the ureteral environment above 37 C shortened the duration of the plateau slightly increased the conduction velocity and increased the spontaneous activity of the ureter Beyond 41° C velocity fell with temperature increase and finally at 45° to 46° C temperatures were reached which produced conductional block A return of temperature to within physiologic limits was followed by a return of conduction if heating beyond the point of block had not been excessive or prolonged

**The Changes in Action Potential Shapes Associated with Healed Ureteral Anastomoses** Analysis of the effect of ureteral transection and anastomosis

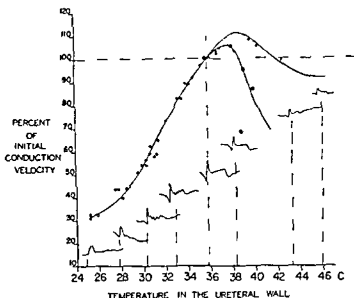



Fig 2 The Effect of Temperature Change Upon Ureteral Peristalsis The diagram shows the effect of various temperatures of the ureteral wall upon wave velocity and action potential shape The lower line of the velocity plot between 42 and 36 indicates the reduced wave velocities accompanying a return of conduction with cooling after conductional block had occurred at higher temperatures A decrease in voltage and a broadening of the peak phase of the action potential accompanied these waves

upon subsequent conduction of peristalsis and its relation to any associated hydronephrosis has been presented[2] The action potentials conducted through the anastomoses and recorded from electrodes placed in the anastomotic lumen showed arrival of the action potential with an approach artefact an iso electric period corresponding to the duration of delay in action potential conduction and a departure spike preceded by a pre potential and followed by an abnormal period of repolarization Table 1 5) The latter consisted of marked oscillations or secondary spikes in place of the plateau of the action potentials These plateau abnormalities were recorded in or adjacent to the ureteral scar in most instances and in all parts of a right ureter studied 2 mo after right nephrectomy ureteral transection and anastomosis (Table 1 6)

**The Effect of Drugs Upon Action Potential Shape** Nicotine curare hexamethonium xylocaine acetylcholine with and without eserine atropine epinephrine and regitine produced no change in velocity of conduction or shapes of the peristaltic action potentials Histamine locally or systemically administered consistently increased the frequency of peristalsis but did not alter significantly the shape of the resulting action potentials Its effect upon conduction velocity consisted of a shortening of the period of reduced wave velocity In other words during the high frequency peristalsis caused by histamine the decrease of velocity caused by the recent passage of a preceding wave was less than normal The administration of tripelennamine citrate (Pyribenzamine) or diphenhydramine hydrochloride (Benadryl) decreased the voltage and broadened the peak phase of both the action

potentials of histamine stimulation and those initiated by distention until they no longer were recordable

DISCUSSION

In this study the factors affecting the conduction of activity were found to be primarily physical ones most drugs tested having no effect upon the shape of peristaltic action potentials Histamine lowered the threshold of conduction in ureteral smooth muscle but did not change the shape of the action potential To date no means of restoring conductivity across ureteral anastomotic scars has been found Antihistaminic drugs such as Benadryl caused a temporary disappearance of ureteral action potentials These findings suggest that antihistaminic drugs may prove useful in the control of ureteral pain Temporary relief of pain has followed the administration of Benadryl in 3 or 4 individuals suffering ureteral colic The effects of histamine and antihistaminic drugs upon ureteral peristalsis suggest that histamine or some histamine like substance may be released by a contracting cell and activate contraction in neighboring cells

REFERENCES

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# THE RENAL LYMPHATICS I THE EFFECT OF DIURESIS AND ACUTE URETERAL OBSTRUCTION UPON THE RATE OF FLOW AND COMPOSITION OF THORACIC DUCT LYMPH*

M K MYINT AND JOHN J MURPHY

Dissection of injected specimens in the dog and in man demonstrate that the major lymphatic channels draining the renal parenchyma make up 6 to 8 trunks surrounding the renal vessels (Fig 1) These trunks empty into the cisterna chyli or thoracic duct usually through 1 or more para aortic lymph nodes Lymph in the cisterna or thoracic duct then should reflect alterations in renal lymph all other conditions being equal

It has been suggested that the renal lymphatics might act as a route of decompression of the renal collecting system when the ureters are obstructed [1] Direct communication between the renal pelvis and lymph channels have also been postulated In an attempt to evaluate these theories and to elucidate the function of the lymphatics in normal and pathologic situations the following experiments were done

*From the Harrison Department of Surgical Research Schools of Medicine University of Pennsylvania Philadelphia Pa

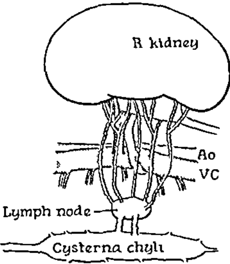



Fig 1 Diagram of anatomy of renal lymphatics

METHOD

Adult mongrel dogs were used as experimental animals and anesthetized with intravenous pentobarbital The thoracic duct was exposed in the right chest and a polyethylene catheter was introduced into the cisterna chyli through the thoracic duct The renal veins and the ureters were exposed transperitoneally Baseline determination of rate of lymph flow and sodium chloride potassium urea nitrogen and total protein content of the lymph were made in all instances Baseline values for these substances in renal and femoral vein blood were also obtained

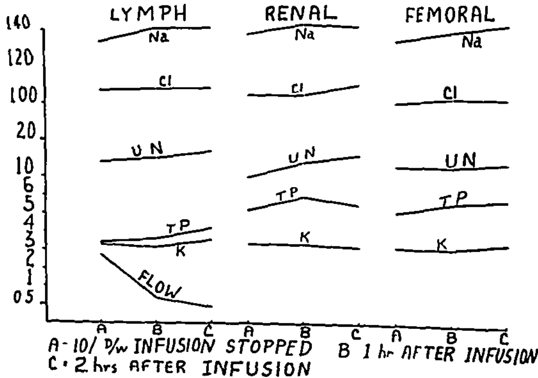



Fig 2 Effect of diuresis alone

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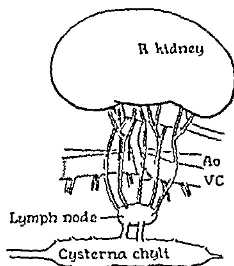



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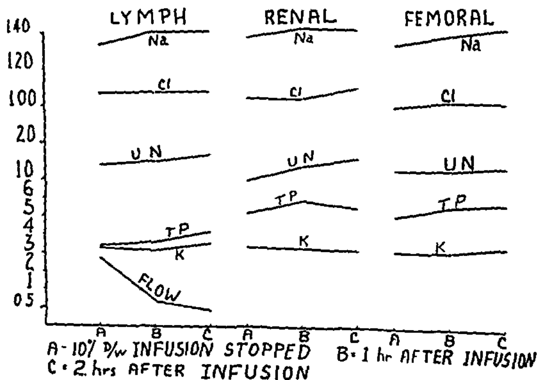



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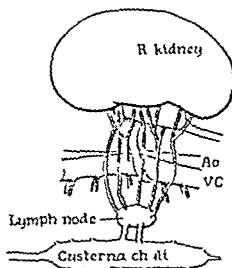



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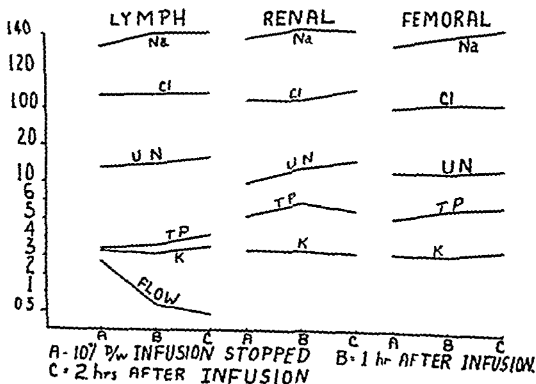



Fig 2 Effect of diuresis alone

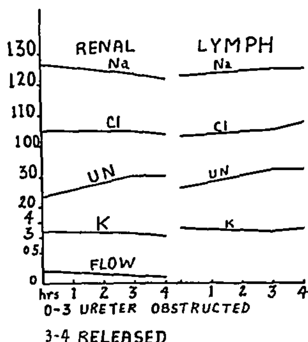



Fig 3 Effect of obstruction of 1 ureter in animal not in diuresis

DISCUSSION

**Controls** Animals in which the usual exposures and visceral manipulation were carried out showed only a transient rise in the rate of lymph flow Values for urea nitrogen total protein sodium potassium and chloride were unchanged after visceral manipulation in the renal and peripheral vein blood as well as in the lymph

**Effect of Diuresis** Diuresis induced by the rapid intravenous administration of from 300 cc to 500 cc of 10 per cent dextrose in water produced an increase in the rate of lymph flow in the cisterna chyli in each of 10 animals (Fig 2) Changes in the concentrations of sodium potassium chloride urea nitrogen and total protein were slight tended to be similar in both blood and lymph and seemed to reflect slight dilution of the intravascular fluids

**Effect of Ureteral Obstruction** In 5 dogs obstruction of a single ureter with normal hydration (not in diuresis) resulted in no significant changes in the rate of lymph flow on the sodium potassium and chloride content of the lymph Urea nitrogen concentration rose slightly during the obstruction in both lymph and renal vein blood (Fig 3)

Obstruction of both ureters during diuresis in 6 dogs produced a significant increase in the rate of flow of lymph (Fig 4) The rate of lymph flow decreased when the diuresis ceased and the obstruction was released Urea nitrogen in lymph renal and femoral vein blood increased during diuresis and obstruction and either leveled off or decreased slightly thereafter Changes in sodium chloride potassium and total protein paralleled each other in lymph and blood and again seemed to reflect the effect of intravascular dilution

The changes observed in lymph flow and composition of lymph in diuresis confirms the impressions noted by Schmidt and Hayman[2] and supports the conclusions of Kaplan[3] that renal lymph is derived from both renal plasma and tubular reabsorbed fluid The failure of unilateral ureteral obstruction

Fig 4 Effect of bilateral ureteral obstruction in presence of diuresis

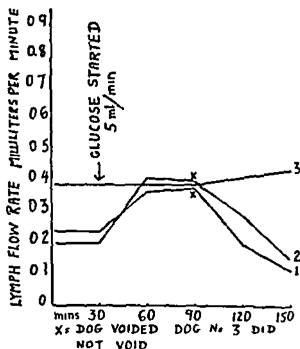



Fig 5 Effect of voiding upon lymph flow

in a dog not in the state of diuresis to produce significant changes in rates of flow and composition of lymph suggest that the safety valve action of the lymphatics in ureteral obstruction is dependent upon definite pressure relationships between the lymphatic channels and the pelvis of the kidney

Thus the intrapelvic pressures which develop under the conditions described here for animals with unilateral ureteral obstruction not in diuresis are probably insufficient to cause pyelolymphatic backflow whereas in those with diuresis and bilateral ureteral obstruction such pressures are developed rapidly and produce the phenomena described

An interesting observation was made during the course of these experiments with diuresis In three animals whose ureters were intact, the bladders were seen to fill rapidly during diuresis While the bladder was filling a rapid rise in the rate of lymph flow was noted in 2 of the animals (Fig 5) After 1 hr the rates of lymph flow remained unchanged for the next half hour whereupon 2 of the dogs voided with resultant rapid fall in the rate of lymph flow over the next hour In the animal which did not void the rate of lymph flow slowly increased The exact significance of this finding is not clear and further investigation is in progress

SUMMARY

1 The rate of lymph flow in dogs is increased by diuresis induced by intravenous glucose in water

2 Obstruction of one ureter in a dog not in a state of diuresis produced no significant change in rate of flow and composition of the lymph

3 Obstruction of both ureters during diuresis produced increase in the rate of lymph formation and changes in the composition of lymph similar to those observed in renal and peripheral vein blood

4 The action of the renal lymphatics as a safety valve appears to be dependent upon definite pressure relationships between the lymphatic channels and the renal pelvis

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2 Schmidt C F and Hayman J M Note upon lymph formation in the dogs kidney and effect of certain diuretics upon it Am J Physiol *91* 157 160 1929

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# THE RENAL LYMPHATICS II. EFFECT OF INCREASING PRESSURE IN THE RENAL PELVIS UPON ABSORPTION OF SUBSTANCES OF VARIOUS MOLECULAR SIZES*

JOHN J. MURPHY AND M. K. MYINT

The fact that material in the renal pelvis may enter the circulatory system under certain conditions is well established. The routes by which transportation through the renal parenchyma is accomplished have been described as pyelo-tubular, pyelo-interstitial, pyelo-venous and pyelo-lymphatic. Evidence for each has been described by radiographic, hydrodynamic and radioactive tracer techniques. The exact role and relative importance of each route in normal and pathological situations is not clear. The experiments described here were designed to elucidate this problem.

## METHOD

Adult mongrel dogs were anesthetized with pentobarbital given intravenously. The thoracic duct was exposed in the right chest, and through it a polyethylene catheter was passed into the cisterna chyli. The ureters were exposed transperitoneally and through them polyethylene catheters were passed to the renal pelvis and secured. A baseline rate of lymph flow was determined and control specimens of lymph, renal and femoral vein blood taken for analysis.

The renal pelves were aspirated and then the pressure within the renal collecting system was gradually increased by adding increments of a solution of the test substance into it through a water manometer. Samples were taken of lymph and blood from the renal and femoral veins at intervals while the pressure was either kept constant or gradually increased. The samples were analyzed for the test substance being used and the results compared with the baseline levels. Graphs have been made with the concentrations of test substances plotted on the ordinate and pressure or time (if pressure was constant) on the abscissa.

## RESULTS

In the first group of animals 50 per cent dextrose in water was introduced into the renal pelvis at 100 cm. of water pressure. The results are shown in Figure 1. The rate of lymph flow doubled in 10 min., then leveled off and slowly increased thereafter up to 70 min. Analysis of the lymph, renal and peripheral blood for glucose showed a marked increase of this material in the lymph in 10 min. and a constant increase thereafter. The renal vein blood showed a definite but slower rise and the peripheral blood slowly reflected these changes.

In another group of animals 50 per cent dextrose in water was introduced at gradually increasing pressures. The increments of pressure ranged from 10 to 20 cm. of water at 10 to 15 min. intervals. Figure 2 demonstrates the results obtained when samples of lymph and blood were taken at 15 to 20 min. intervals during this experiment and analyzed for glucose. It is apparent that the glucose concentration of the lymph began to rise sharply at

* From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia, Pa.

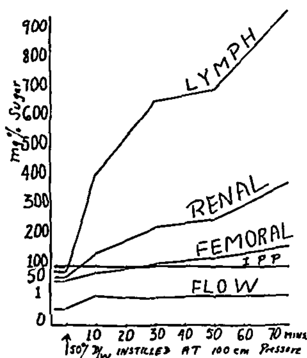



Fig. 1 Effect of 50 per cent D/W at 100 cm water pressure in renal pelvis upon rate of flow of lymph and concentration of glucose in lymph and blood from renal and femoral veins

30 cm of water pressure while in the renal vein such a rise was not seen until after 40 cm of water pressure was reached The curve representing glucose concentration in the lymph tends to parallel that of the pressure while the renal vein blood concentration level off at 55 cm of water pressure

In order to evaluate the transportation of a slightly larger molecule the dye T 1824 (molecular weight of about 980 as compared with glucose of

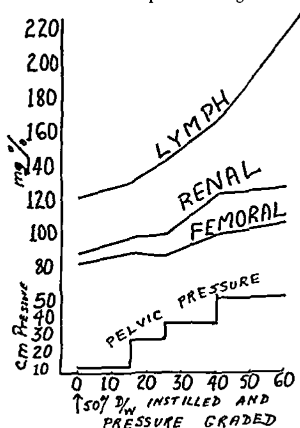



Fig 2 Effect of 50 per cent D/W instilled into the renal pelves at gradually increasing pressure upon glucose concentration in lymph and renal and femoral vein blood

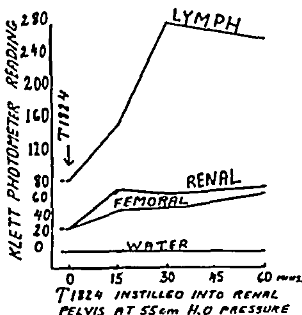



Fig 3 Photometric comparison of lymph and of plasma from the renal and femoral veins while the renal pelves contain T 1824 at 55 cm $H_2O$ pressure

180) was chosen This substance can easily be identified visually and the concentration measured photometrically Instillation of a solution of the dye into the renal pelvis at 55 cm of water pressure yielded some dramatic evidence regarding modes of transport from renal collecting system to the circulatory system

Gross examination of the lymph obtained from animals during such an experiment clearly demonstrates the presence of the dye in increasing concentrations after 15 min Visual comparison of samples from the cisterna renal and femoral veins up to 30 and 60 min leaves no doubt that the lymph contains much more of the dye than does the plasma

A more exact comparison of dye concentration in such an experiment is shown in a graph The Klett photometric reading of the lymph and plasma is plotted on the ordinate with time in minutes on the abscissa (Fig 3) Here again it is of interest that the concentration of T 1824 in the renal vein levels off after 15 min while that in the lymph continues to rise up to ½ hr

Examination of a kidney sectioned after such an experiment demonstrates the dye beautifully outlining the renal pyramid and here and there extending through the cortex to the capsule This is graphic evidence for the pyelotubular and pyelointerstitial pathways When combined with the evidence previously presented this makes a strong argument for the importance of the lymphatic route of transfer from the pelvis through the tubules and interstitial tissues to the circulatory system when increased intrapelvic pressures exist

## SUMMARY AND CONCLUSIONS

When introduced into the renal pelvis under pressure glucose and the dye T 1824 appear in lymph of the cisterna chyli earlier and in greater concentration than in the renal or femoral vein blood

The appearance of the dye T 1824 in the renal tissues after its introduction into the pelvis in these experiments suggests that an important route of transfer under these conditions is from the pelvis through tubules and interstitial tissues and thence through the renal lymphatics into the circulatory system

# A STUDY OF URETEROSIGMOIDOSTOMY IN THE DOG WITH AND WITHOUT DIVERTING COLOSTOMY*

J S Ansell and C D Creevy

The problem of renal insufficiency following urinary diversion is often attributed to ascending infection of the transplanted ureter Infection may be the result of continual bacterial contamination and reflux from the bowel In other instances scarring of the terminal ureter and of the intestinal wall through which it passes leads to stricture formation and secondarily infected hydronephrosis Since Hinman s[1] masterful review of the subject new modes of urinary diversion have been described Nesbit[2] advocated a wide spatulate end to side union between ureter and intestine with no attempt to prevent reflux Leadbetter[3] suggested a spatulate mucosa to mucosa anastomosis plus a sharply dissected submucosal trough for the terminal ureter Goodwin[4] fashions a similar trough by blunt dissection from within the bowel outwards Ssolowoff[5] suggests a tunnel for the terminal ureter made like that formed by Witzel around a gastrostomy tube Mathison [6] after studying avian and reptilian cloacae described a method of wrapping a full thickness flap of colon around the terminal ureter as it pierces the bowel to form an intra luminal nipple to prevent reflux The use of diverting colostomy to prevent continued resoiling of transplanted ureters by the fecal stream has been suggested in clinical problems by the senior author [7] and Vest[8] recently and others in the past

## METHOD

This study surveys the above methods of ureterocolic anastomosis in the dog with and without diverting colostomy Bilateral ureterosigmoidostomies were fashioned in 35 mongrel dogs of mixed sexes Twenty of these dogs had diverting end colostomies The proximal end of the distal colon was then closed and dropped back into the peritoneal cavity to provide a site for anastomosis and act as a reservoir for urine In order to avoid influence of anatomical position on the results the various types of anastomoses were alternated from left to right side according to a predetermined plan Dogs were fed low residue diets in the 48 hr before operation and given 2 0 gm Neomycin and 0 4 gm Polymyxin B orally in 4 divided equally spaced doses Penicillin 500 000 units and 0 5 gm Streptomycin were injected intramuscularly twice daily during the first 4 days after operation Distribution of anastomosis is shown in Table 1

One dog died with distemper on the first postoperative day Another died of aspiration pneumonitis on the fourth day after operation A third died of infarction of the proximal portion of the colonic reservoir down to and including the anastomotic sites A fourth dog died on the thirty second postoperative day with intussusception of the colonic reservoir These 4 animals are included in the mortality for the series but not in the distribution table nor in subsequent statistics compiled on the basis of the length of time the urinary tract remained normal A fifth dog died on the first postoperative day with a leak from a Goodwin anastomosis The Mathison on the contra

*From the Departments of Surgery Divisions of Urology of the University of Minnesota Hospitals and the Minneapolis Veterans Administration Hospital

*Table 1 Distribution of Anastomoses*

| DOG | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Colostomy | + | — | + | — | + | + | + | — | — | + | + | — | — | + | + | + | — | + | — | — | + | + | + | — | + | + | — | — | + | — | + |
| Leadbetter | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | | | | | | | | | | | | | | ✓ | ✓ |
| Leadbetter | | ✓ | ✓ | ✓ | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Witzel | | | | | | | | | ✓ | | | | | | | | | | | | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Mathisen | ✓ | | | | X | ✓ | ✓ | | | | ✓ | ✓ | ✓ | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | |
| Goodwin | | | | | | | | | | | | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | | | |
| Nesbit | ✓ | | | | | | | | | | | | | ✓ | ✓ | ✓ | | | | | | | | | ✓ | | | | | | |

Table 1 This table shows distribution of types of anastomoses in each animal. With the exception of the 3 animals which had Leadbetter anastomoses bilaterally each dog had a different type of anastomosis performed on each ureter

lateral side was not included in the duration normal statistics All dogs dying in the 6 wks following operation were considered operative deaths These were the above described making the operative mortality 11 3 per cent

All dogs were followed postoperatively by monthly checks of blood urea nitrogen carbon dioxide chloride sodium and potassium At intervals of 3 mo each animal had an excretory urogram and a contrast study of the colon

All animals but 1 were acidotic prior to death and all but 3 were hyperchloremic. The final blood urea nitrogen averaged 52 5 mg per cent $CO_2$ combining power 18 chlorides 121 sodium 146 and potassium 4 1 mEq each There was no significant difference in blood chemistries between animals with and without colostomy

Results tabulated according to the length of time the upper urinary tract remained normal as demonstrated by urography and post mortem examination show that collecting systems transplanted after the method of Leadbetter remained normal an average of 289 days (range 0–800) Goodwins 170 days (range 12–468) Mathisons 138 days (range 0–513) Nesbit s 84 days (range 12–232) and Witzel s 77 days (range 0–253) In dogs with colostomies the urinary tracts remained normal an average of 194 days without colostomies 196 days The 2 dogs which survived longest 884 and 818 days had colostomies In almost every instance there was histological

*Table 2*

| DOG | COLO | DAYS GROSSLY NORMAL | | | FINAL CHEMISTRIES | | | |
|---|---|---|---|---|---|---|---|---|
| | | LEADBETTER | MATHISON | BUN | $CO_2$ | Cl | Na | K |
| 193 | + | 404 | 120 | 13 | 20 | 118 | 153 | 4 6 |
| 220 | + | 343 | 30 | 9 | 14 | 124 | 144 | 4.3 |
| 222 | + | 800 | 513 | 38 | 17 | 118 | 144 | 4 3 |
| 230 | — | 44 | 20 | 39 | 21 | 114 | 150 | 3 7 |
| 234 | — | 225 | 469 | 53 | 23 | 103 | 146 | 3.2 |
| 236 | + | 464 | 464 | 26 | 12 | 128 | 116 | 4 7 |
| 237 | + | 52 | 52 | 181 | 13 | 132 | 141 | 3.5 |
| 238 | — | 789 | 33 | 300 | 7 | 118 | 155 | 4 3 |
| 239 | — | 743 | 114 | 41 | 16 | 124 | 143 | 4 5 |
| Average | | 407 | 205 | 44 | 16 | 120 | 147 | 4 0 |

evidence of pyelonephritis in the post mortem material examined Tables 2 and 3 show some comparisons between Leadbetter Goodwin and Mathison anastomoses Table 4 shows the number of days urinary tracts remained grossly normal for all animals

*Table 3*

| DOG | COLO | DAYS GROSSLY NORMAL GOODWIN | MATHISON | FINAL CHEMISTRIES BUN | CO | Cl | Na | K |
|---|---|---|---|---|---|---|---|---|
| 141 | − | 378 | 0 | 34 | 18 | 121 | 152 | 4 8 |
| 209 | + | 53 | 53 | 27 | 23 | 127 | 156 | 5 2 |
| 228 | + | 272 | 184 | 28 | 25 | 117 | 156 | 3 9 |
| 244 | − | 468 | 90 | 65 | 15 | 126 | 149 | 4 4 |
| 249 | − | 66 | 66 | 16 | 19 | 114 | 141 | 4 1 |
| 251 | + | 180 | 180 | 27 | 18 | 126 | 149 | 3 7 |
| 280 | + | 73 | 73 | 75 | 12 | 132 | 161 | 5 3 |
| 242 | − | 0 | | | | | | |
| Average | | 186 | 92 | 39 | 19 | 127 | 152 | 4 5 |

*Table 4*

| | DAYS GROSSLY NORMAL LEADBETTER + | − | GOODWIN + | − | WITZEL + | − | NESBIT + | MATHISON + | − |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 281 | 53 | 378 | 60 | 62 | 47 | 60 | 162 |
| | 232 | 400 | 272 | 62 | 110 | 12 | 232 | 120 | 0 |
| | 404 | 225 | 468 | 66 | 253 | 44 | 46 | 53 | 12 |
| | 313 | 44 | 180 | — | | 0 | 54 | 30 | 469 |
| | 800 | 587 | | | | | 42 | 513 | 33 |
| | 164 | 743 | | | | | | 184 | 114 |
| | 0 | 60 | | | | | | 164 | 90 |
| | 110 | 46 | | | | | | 0 | 66 |
| | 62 | 463 | | | | | | 180 | 73 |
| | 62 | 463 | | | | | | 54 | |
| | | | | | | | | 17 | |
| Av | 248 | 331 | 324 | 169 | 141 | 39 | 84 | 158 | 113 |

Table 4 Days collecting system remained grossly normal as determined by Excretory Urography or Post Mortem examination
+ —with diverting colostomy
− —without diverting colostomy

## CONCLUSIONS

We conclude on the basis of these results that the difference between various techniques of uretero colic anastomosis in the dog seems to be a quantitative rather than a qualitative one When the ureter is transplanted to colon in the dog with or without *diversion of the fecal stream* ascending infection and renal failure eventually ensue

The colon of the dog has no taenia 3 muscular layers and a plastic consistency that is quite different from the human colon The anatomy of the canine colon may have had considerable influence on the results of this study

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# OBSERVATIONS ON THE OPERCULUM TECHNIQUE FOR URETEROENTEROSTOMY*

Jack Lapides John M Bobbitt James W Morrow and Russell Scott Jr

Although the majority of patients with bilateral ureterosigmoidostomy develop eventually pyelonephritis hydronephrosis and hyperchloremic acidosis a small number remain perfectly normal for periods as long as 20 yr or more Sigmoidoscopic examination of one of these normal patients revealed the ureteral orifices to be covered by overlying polypoid structures apparently arising from intestinal mucosa It was theorized that perhaps these mucosal flaps accounted for the normal upper urinary tract by preventing reflux of fecal material up the ureter and thus minimizing pyelonephritis In view of this consideration it was decided to develop a surgical technique whereby rectal mucosal flaps would be created to cover the ureteral orifice like an operculum

## Method

The technique devised to create flaps of rectal mucosa is illustrated in Figure 1 The sigmoid colon is incised in a longitudinal manner along the antimesenteric border and a flap of mucosa is dissected free in the posterolateral region of the bowel lumen as shown in Figure 1 (1) The flap is about 1 5 cm long by 1 cm wide its free edges are approximated with fine catgut to form a tit (Fig 1 (2)) A small hemostat is then pushed directly through the bowel wall at the base of the flap the ureter grasped and drawn into the bowel lumen (Fig 1 (3)) The distal end of the ureter is spatulated and its free

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evidence of pyelonephritis in the post mortem material examined Tables 2 and 3 show some comparisons between Leadbetter Goodwin and Mathison anastomoses Table 4 shows the number of days urinary tracts remained grossly normal for all animals

*Table 3*

| DOG | COLO | DAYS GROSSLY NORMAL GOODWIN | MATHISON | FINAL CHEMISTRIES BUN | CO | Cl | Na | K |
|---|---|---|---|---|---|---|---|---|
| 141 | − | 378 | 0 | 34 | 18 | 121 | 152 | 4 8 |
| 209 | + | 53 | 53 | 27 | 23 | 127 | 156 | 5 2 |
| 223 | + | 272 | 184 | 28 | 25 | 117 | 156 | 3 9 |
| 244 | − | 468 | 90 | 65 | 15 | 126 | 149 | 4 4 |
| 249 | − | 66 | 66 | 16 | 19 | 114 | 141 | 4 1 |
| 251 | + | 180 | 180 | 27 | 18 | 126 | 149 | 3 7 |
| 280 | + | 73 | 73 | 75 | 12 | 132 | 161 | 5 3 |
| 212 | − | 0 | | | | | | |
| Average | | 186 | 92 | 39 | 19 | 127 | 152 | 4 5 |

*Table 4*

| | DAYS GROSSLY NORMAL LEADBETTER + | − | GOODWIN + | − | WITZEL + | − | NESBIT + | MATHISON + | − |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 281 | 53 | 378 | 60 | 62 | 47 | 65 | 162 |
| | 232 | 400 | 272 | 62 | 110 | 12 | 232 | 120 | 0 |
| | 404 | 225 | 468 | 66 | 253 | 44 | 46 | 53 | 12 |
| | 343 | 44 | 180 | − | | 0 | 54 | 30 | 469 |
| | 800 | 587 | | | | | 42 | 543 | 33 |
| | 464 | 743 | | | | | | 184 | 114 |
| | 0 | 60 | | | | | | 464 | 90 |
| | 110 | 46 | | | | | | 0 | 66 |
| | 62 | 463 | | | | | | 180 | 73 |
| | 62 | 464 | | | | | | 54 | |
| | | | | | | | | 17 | |
| Av | 218 | 331 | 324 | 169 | 141 | 39 | 81 | 158 | 113 |

Table 4 Days collecting system remained grossly normal as determined by Excretory Urography or Post Mortem examination
+ —with diverting colostomy
− —without diverting colostomy

CONCLUSIONS

We conclude on the basis of these results that the difference between various techniques of uretero colic anastomosis in the dog seems to be a quantitative rather than a qualitative one When the ureter is transplanted to colon in the dog with or without diversion of the fecal stream ascending infection and renal failure eventually ensue

The colon of the dog has no taenia 3 muscular layers and a plastic consistency that is quite different from the human colon The anatomy of the canine colon may have had considerable influence on the results of this study

visualization on one side Autopsy revealed the absence of all the nipples except in one dog who had a short fibrotic tit The ureteral orifices were patent in all animals with the exception of the dog demonstrating non visualization on one side this animal had a complete stenosis of the ureteral opening Although 11 of the 12 kidneys appeared to be grossly normal microscopic examination demonstrated chronic pyelonephritis to be present in all of them

### DISCUSSION

On the basis of the present experimental observations it would appear that mucosal flaps remain viable but disappear over a prolonged period of time due to retraction rather than atrophy It is interesting to note that although the pyelograms appeared to be perfectly normal hyperchloremic acidosis was present associated with necropsy findings of chronic pyelonephritis

### SUMMARY

1 Intestinal mucosal flaps were constructed in dogs to act as flutter valves over the ends of the ureters this was done in an attempt to prevent fecal reflux with resultant pyelonephritis and hyperchloremic acidosis

2 The results of these studies reveal that the mucosal flaps remain viable for 2 to 4 wks but disappear after 6 mo probably due to retraction

3 Despite normal pyelograms hyperchloremic acidosis and chronic pyelonephritis were present in most of the dogs

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## EXPERIMENTAL STUDY OF THE REGENERATION OF THE BLADDER IN DOGS*

M K Than J Brunson and C D Creevy

Although sporadic clinical observations have been made by Richardson[1] Folsom[2] and others that the bladder will regenerate after subtotal cystectomy extensive clinical application has not been made of this principle primarily because of the more popular techniques of diverting the urine into the intestinal tract after excision of the bladder Experimentally the work of Berry[3] Schuller[4] and Kretschmer[5] has shown that the bladder will regenerate in experimental animals after subtotal cystectomy Bohne was the first to prove that the bladder will regenerate after total cystectomy Little follow up has been available on clinical cases and the phenomena of bladder regeneration has been observed in only a limited number of animals The purpose of this experiment has been to study regeneration of the bladder more specifically both from a histologic and from a functional standpoint

These experiments show that regeneration of the canine bladder does

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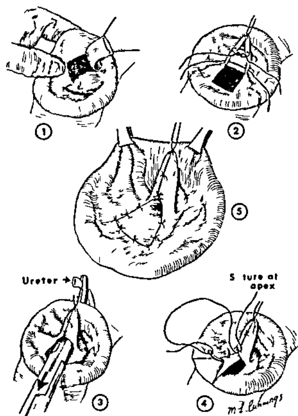



Fig 1 (1) Rectangular flap of mucosa dissected free (2) Approximation of edges of flap to form a tit (3) Grasping ureter to pull through bowel wall at base of operculum (4 5) Anastomosis of spatulated ureter to intestinal mucosa

edges sewn to the incised borders of the intestinal mucosa as well as to the base of the tit as demonstrated in Figures 1 (4) and 1 (5) The same method was utilized also to create flaps of bladder mucosa

Initially dogs were autopsied 2 and 4 wks postoperatively to ascertain viability of the mucosal flaps When it was established that the flaps remained viable unilateral reimplantation of the ureter into the bladder of each of 3 female dogs was performed Pyelograms and cystograms were obtained 3 mo after operation The results of these urograms stimulated the performance of the operculum type ureterosigmoidostomy bilaterally in 10 dogs All of these dogs were followed with interval blood chemistry studies and pyelography

The animals with reimplantation of one ureter into the bladder were sacrificed at the end of 13 mo Three dogs with bilateral ureterosigmoidostomy were sacrificed at the end of 6 mo and 3 more at the end of 11 mo Autopsies were conducted on all the animals

## RESULTS

The urograms on the animals with unilateral reimplantation of the ureter into the bladder revealed normal pyelograms with no evidence of ureteral reflux At autopsy examination of the bladder disclosed that the mucosal flaps had disappeared Microscopic examination of the kidneys on the transplanted side revealed chronic pyelonephritis

Blood chemistry studies on the dogs with bilateral ureterosigmoidostomy showed moderate hyperchloremic acidosis with normal blood urea nitrogen levels Most of the pyelograms demonstrated normal collecting systems However one dog showed minimal hydronephrosis while another had non

Three months after the initial operation blood chemistry and hemoglobin determinations were again made If found normal an excretory urogram and cystogram was made and the capacity of the bladder was measured Dogs were followed at 3 mo intervals thereafter with urograms cystograms blood chemistry determinations and bladder capacity measurements To observe the stages of regeneration histologically some dogs were sacrificed at intervals of 2 to 4 wks

## RESULTS

In all 42 animals were used Table 1 shows the fate of all the animals Two died within 24 hr following initial operation from leakage of urine into the peritoneum One died 24 hr after the second operation due to anesthesia and 15 died of urinary obstruction and infection Four of these also had distemper All of these 15 dogs succumbed within 2 to 5 wks after excision of the bladder At autopsy they had (1) pressure necrosis of the trigone from the prosthesis and (2) hydroureter and hydronephrosis from obstruction of the intramural ureter Of the remaining animals 14 were sacrificed at intervals and will be discussed later Ten are still under observation having now survived 6 to 12 mo following the initial operation

As mentioned above 14 dogs were sacrificed at intervals after the initial operation 2 each at the 2nd 4th 8th, 12th 14th 20th and 24th wks The results of gross and microscopic study are as follows

(1) At 2 wks postoperative the prosthesis was surrounded by an inflammatory mass which upon microscopic examination consisted of acute inflammatory cells predominantly polymorphonuclear neutrophiles

(2) At 4 wks there was a rather thick walled inflammatory mass containing granulation tissue and many capillaries

(3) At 8 wks there was globular mass with a smooth glistening inner surface The outer surface was adherent anteriorly to the rectus sheath with some adhesions laterally and posteriorly to adjoining tissues On microscopic examination the pouch was now lined by transitional epithelium

(4) At 12 to 14 wks after operation the gross appearance differed little from the preceding but microscopic examination disclosed an inner layer of transitional epithelium with a base of granulation tissue surrounded by a zone of fibroblasts lymphocytes and fibrous tissue intermingled with smooth muscle

(5) At 20 to 24 wks after operation there was a pouch about 10 mm in thickness with smooth internal surface Microscopically there was a multilayered inner zone of transitional epithelium and a middle zone of submucosal connective tissue This was surrounded by a layer of smooth muscle which was thicker than before and which was covered externally by a layer of fatty tissue

Van Gieson s stain colored the smooth muscle yellow and the connective tissue deep crimson but did not reveal ganglion cells or other nerve tissue

Follow up Intravenous urography was carried out on 28 dogs Ninety per cent of the dogs revealed slight hydro-ureter at the first examination about 4 wks postoperatively presumably due to compression of the trigone by the prosthesis since it decreased after removal of the latter Ten per cent had persistent hydroureter Cystography in 16 dogs revealed a globular pouch There was ureteral reflux in only one animal in which the ureter was inad

occur that it is formed of smooth muscle lined by transitional epithelium and, although of limited capacity is capable of contracting at intervals and *of emptying itself*

METHOD

Adult mongrel female dogs weighing 15 kg or more were used Nembutal 13 mg /kg body weight was used for intravenous anesthesia Through a sub umbilical median incision the bladder was exposed transperitoneally It was dissected as widely as possible from the peritoneum which was closed with 00 chromic catgut to isolate the peritoneal cavity from the bladder area The bladder was opened on its anterior aspect and the ureteral orifices iden tified Number 4 French ureteral catheters were passed up the ureters and the bladder was resected leaving only the trigone Bleeding around the cut edges of the trigone was controlled with 3 0 chromic catgut and the ureteral catheters were removed A polyethylene ball with multiple 5 mm perfora tions was placed over the trigone The prosthesis was covered with peri toneum and perivesical areolar tissue sutured to the cut edges of the trigone and internal urinary meatus A stab was made over one of the holes of the prosthesis and a number 22 Malecot catheter was introduced through it into the lumen of the ball and led out through a separate stab wound in the abdominal wall A nylon guard, to prevent the catheter from being bitten was threaded over the catheter which was anchored to the anterior rectus sheath with number 30 steel wire The wound was closed in layers and the catheter was cut flush with the external opening of the nylon guard

Four hundred thousand units of penicillin were given immediately after operation and twice daily for 3 days The dogs were under observation for periods up to 12 mo Four to 5 wks after operation the urea nitrogen carbon dioxide combining power serum chlorides total serum proteins and hemo globin were measured If results were within normal limits an excretory uro gram was made under anesthesia the following day *At the same time the* polyethylene prosthesis was removed through a small incision The bladder area was examined and irrigated thoroughly with normal saline and solu tion M* to wash out all the necrotic debris and soft sand No attempt was made to divide the adhesions on its external surface The pouch was then *closed with interrupted 000 chromic catgut sutures The rectus muscles and* skin were approximated with No 30 steel wire Intensive antibiotic therapy consisted of 400 000 units of penicillin twice daily for 1 wk followed by 1 gm of gantrisin orally per day for another week

*Table I*

| | |
|---|---|
| No of dogs operated upon | 42 |
| No of dogs died 24 hr P O | 2 |
| No of dogs died 24 hr after second operation | 1 |
| No of dogs died from 2 to 5 wks | 15 |
| No of dogs sacrificed | 14 |
| No of dogs still alive | 10 |
| Total | 42 |

*Solution M pH 4.5 Citric acid monohydrate 32 5 gm magnesium oxide anhydrous 3 84 gm sodium carbonate 8 84 gm distilled water 1000 cc

dog had a BUN of 60 mg which came down to 10 mg 3 days after removal of the ball and fluid therapy The urea nitrogen was 20 mg 6 mo after the initial operation Of the 15 dogs that died the urea nitrogen was measured in 5 and averaged over 60 mg per cent

## DISCUSSION

The difficulty of training the dogs not to bite the suprapubic tube has been solved by putting a nylon guard over the catheter Urinary tract infection and stone formation has been a major problem because of the open suprapubic tube drainage However if the bladder was washed thoroughly with saline and solution M at the secondary operation followed by an intensive course of antibiotics stones did not recur The capacity of the bladder although not great did not decrease with prolonged observation This study suggests that clinical application might be made of this principle in suitable cases Such a method would be superior to urinary diversion for the following reasons it is a less extensive operation the intramural portion of the ureter is not disturbed hence reflux is prevented and also the ureters do not come in contact with the intestinal contents It is suggested that this method might be applicable in the treatment of extensive trauma of the urinary bladder following excision of localized carcinomas in exstrophy and following excision of the bladder for interstitial cystitis

## SUMMARY AND CONCLUSIONS

Regeneration of the canine urinary bladder has been studied following excision of the bladder save for the trigone and ureteral orifices Early and late deaths occurred in 42 8 per cent of the dogs Study of the remainder has shown that regeneration of a functioning bladder occurred in 75 per cent Histological and functional studies of the regenerated bladder have been discussed

## REFERENCES


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3 Berry F The regeneration of smooth muscles J Med Res *41* 365 1920
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Fig 1 Photomicrograph of regenerated bladder of dog 12 wks after initial operation There is an inner layer of transitional epithelium below which is a zone of fibroblasts and smooth muscles



Fig 2 Cystogram of dog 6 mos following initial operation Note the globular smooth outline There is no reflux



Fig 3 Specimen of dog sacrificed 6 mo after initial operation The inner surface of the bladder is smooth and glistening This pouch was 10 mm in thickness Note the normal upper urinary tract

vertently severed during excision of the bladder The original capacity of the new bladder ranged from 30 to 40 cc In 10 dogs not sacrificed the bladder was stretched weekly by cautious over distension An average final capacity of 60 cc was obtained in 16 dogs with a range of 40 to 110 cc The animals did not dribble were continent and voided with a good stream

BUN $CO_2$ and chlorides were not much disturbed in 24 dogs Only one

dog had a BUN of 60 mg which came down to 10 mg 3 days after removal of the ball and fluid therapy The urea nitrogen was 20 mg 6 mo after the initial operation Of the 15 dogs that died, the urea nitrogen was measured in 5 and averaged over 60 mg per cent

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# TOTAL MUCOSAL DENUDATION OF THE CANINE BLADDER EXPERIMENTAL OBSERVATIONS AND CLINICAL IMPLICATIONS*

*Preliminary Report*

ARNOLD R. SANDERS, CLARENCE J. SCHEIN AND LAZARUS A. ORKIN

Recent studies in this laboratory have demonstrated the remarkable proliferative capacities of uroepithelium.[1, 2] When autogenous arterial grafts were substituted for 10 cm. segments of the canine ureter, the graft endothelium was replaced by transitional cell epithelium within 6 wks. This epithelialization traversed the anastomoses from the intact ureter to line the entire graft. Since transitional cell epithelium did proliferate across defects of such length, a series of experiments was undertaken to determine whether it would reline the bladder of the dog whose mucosa had been completely removed and to evaluate the morphological and functional effects of such denudation upon the canine urinary tract.

## METHOD

Male and female mongrel dogs ranging in weight from 17 to 35 lbs. were utilized without special preoperative medication. A standard veterinarian solution of nembutal was administered intravenously for anesthesia. The lower abdomen was opened through a left rectus muscle splitting incision and the urinary bladder was delivered onto the abdominal wall. The bladder dome was incised between Allis clamps and the incision was extended on the ventral surface down the neck.

A small wheal was raised in the mucosa at the superior edge of the opened bladder by the submucosal injection of saline which was then extended to cover the entire surface of the bladder by the further injection of saline. This converted the mucosa into a large bulla. A plane of cleavage was easily developed between the elevated mucosa and the muscularis and the mucosa was completely stripped from the underlying detrusor by blunt and sharp dissection. Hemostasis was obtained by the application of pressure to the denuded surface.

The bladder was closed around a #20 F. Malecot tube with a continuous catgut suture. Penrose drains were inserted into the prevesical space. The abdominal wall was closed in layers, carefully extraperitonealizing the bladder stoma.

All animals were operated upon in this manner with one exception. In this dog a 2 mm. cuff of mucosa was left intact around the left ureteral orifice.

Each dog received 1000 cc. of 5 per cent glucose in water intravenously during the operation and 300,000 u. of penicillin and 0.5 gm. of streptomycin daily for 1 wk. after surgery. The tube and Penrose drains were removed on the seventh postoperative day. Serum sodium, potassium, CO2, chlorides and blood urea nitrogen determinations were obtained on the

*From the Surgical Research Laboratory and the Urological and General Surgical Services, Montefiore Hospital, New York City. Aided by a grant from The Simon Holland Research Fund.

third seventh fourteenth and twenty first postoperative days Intravenous urograms were taken postoperatively at 2 wk intervals for the first month after surgery and at monthly intervals thereafter Retrograde cystograms were obtained at monthly intervals after the cystostomy sinus was completely closed and dry

A total of 8 dogs was utilized for these studies Two animals died 1 on the third postoperative day 1 from bronchopneumonia and severe gastroenteritis The second dog expired on the fourth postoperative day due to a technical error In this case the cystostomy site had not been adequately extraperitonealized and urinary leakage eventuated in a generalized peritonitis The remaining animals tolerated bladder denudation extremely well They ate and drank with zest the morning following operation and showed no ill effects from the procedure Urinary drainage was free of gross b... within 72 hr and the cystostomy sinuses closed within 10 to 12 days a... removal of the tube The animals apparently encountered no di... voiding thereafter and by the fifth week after surgery their urines were ... and free of infection Four dogs have been sacrificed to obtain ana... histologic data The remaining 2 alive and well at present are be... lowed to evaluate the long term results of the procedure

**Blood Chemistries** A mild temporary elevation in blood urea ni... noted in all animals for the first week after operation but this ... normal by the fourteenth postoperative day Other blood ch... mained unchanged from preoperative levels

**Intravenous Urography** In 2 dogs the upper urinary trac... graphically normal at all times Initial postoperative excretory ... the remaining animals disclosed the presence of varying d... urinary tract dilatation ranging from a mild uretercta... hydroureter and hydronephrosis However, this dilata... and in all cases reverted to a normal radiographic patter... (Fig 1)



Fig 1A Excretory urogram ... Left side shows mild pyelectasis ... pyelo calyectasia Both lower ... capacity decreased B Excre... operation Ureteral dilatation ... tract dilatation markedly d... gram 60 days after opera... Note improvement in ...

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The bladder was closed around a #20 F Malecot tube with a continuous catgut suture Penrose drains were inserted into the prevesical space The abdominal wall was closed in layers carefully extraperitonealizing the bladder stoma

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Fig 3 Photomicrograph of re epithelialized bladder 238 days after operation Section taken from retrotrigonal area (Hematoxylin and eosin or iginal X200)

dense connective tissue fibrils blood vessels and a round cell infiltrate (Fig 3) Sections obtained from the dome and lateral walls of the bladder revealed a thinner epithelium with a more edematous submucosa prolif erating fibroblasts numerous small blood vessels and fewer round cells

The thickening of the vesical wall on gross examination seemed attribu table to the changes noted in the submucosa The muscularis and adventitia were not remarkable

DISCUSSION

Total vesical denudation is a simple technical procedure which is well tolerated by the dog A new uroepithelium derived from the ureteral and urethral stumps relines the bladder within 16 wks There is no significant sacrifice of capacity Postoperative upper urinary tract dilatation and reflux appear to be transitory and can be avoided entirely by retaining a small cuff of vesical mucosa around the ureteral orifices These complications in the dog have been observed in this laboratory after other types of vesical surgery involving the trigone and may represent a species peculiarity

That transitional cell epithelium would reinvest the totally denuded blad der is not surprising Numerous investigators have demonstrated the remark able proliferative capacities of uroepithelium in relining grafts used as ureteral and vesical substitutes Bohne Osborne and Hettle replaced the en tire canine bladder with an acrylic mold[3] A pouch like substitute bladder was formed within 4 mo completely lined by a uroepithelium derived from the intact ureters and urethra

Clinically total mucosal denudation might be applicable to the human with recurrent multiple papillomata of the bladder or multiple papilloma tosis Present day treatment of these growths is disappointing Recurrence with eventual malignant degeneration and extension takes place all too fre quently Whether such tumors recur because of contact with the parent growth arise independently from different foci within a tumor prone blad der or are the result of repeated exposures to known or unknown car cinogens is still controversial However their malignant potential is uni versally recognized and short of partial or total cystectomy their long term management has not been too successful

Draper Stark and Lau have suggested the replacement of the mucosae

**Retrograde Cystography** Initial retrograde cystography, performed on the 60th postoperative day disclosed the presence of ureteral reflux in all but one animal The single exception was the ureter about whose orifice a 2 mm rim of mucosa had been left intact Ureteral reflux was never demonstrable in this case In 2 dogs subsequent periodic retrograde cystograms revealed a complete disappearance of reflux In the others reflux persisted but with a marked diminution of the upper urinary tract dilatation noted on earlier studies

Initial retrograde cystograms also demonstrated a moderate loss in bladder capacity estimated at 3 to 4 oz However periodic retrograde cystograms thereafter showed progressive improvement and on the final study vesical capacity ranged from 8 to 10 oz No evidence of bladder contracture was encountered in any animal

**Post Mortem Findings** Examination of the 2 dogs which expired in the first week after operation disclosed mild dilatation of the ureters but normal kidneys The bladder was congested and edematous in each animal Its luminal surface was markedly irregular and covered with a gray exudate

Dogs were sacrificed at 112 120 232 and 268 days after operation In all instances the ureters and kidneys appeared grossly normal No ureteral nor pelvi calyceal dilatation existed anatomically Although the vesical walls revealed mild to moderate thickening the bladders were not contracted In each animal the luminal surface was pink smooth glistening and appeared to be completely re epithelialized (Fig 2) No encrustations nor calculi were found

**Histologic Specimens** Tissue sections demonstrated that the urinary bladder was completely re epithelialized within 16 wks The new mucosa appeared normal in respects and varied from 3 to 12 or more cell layers in thickness Sections taken close to the ureteral orifices or urethra showed a greater depth of uroepithelium with a compact submucosa consisting of

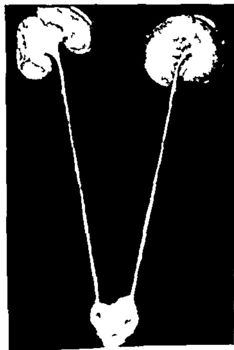


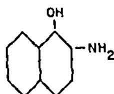
Fig 2 Gross specimen 238 days after operation Surface of bladder smooth glistening and re epithelialized

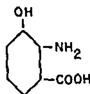
# INVESTIGATIONS OF POSSIBLE ENDOGENOUS CARCINOGENS*

JEFFREY G WATSON

This is a presentation of one facet of an investigatory program that is being carried out to determine the relationship of carcinogens and bladder cancer The origin of this work began at the Chester Beatty Research Institute London England and one phase of it was continued in the Section of Urology of the Department of Surgery University of Michigan

We investigated the possible carcinogenic properties of breakdown products of the amino acid tryptophan because we think that such products in the urine of man may be a cause of bladder cancer These products especially 3 hydroxyanthranilic acid have a similar chemical structure to the known industrial carcinogen 2 amino 1 naphthol the urinary carcinogen of 2 naphthylamine Bonser[1] has shown that the conjugated form of




2-AMINO-I-NAPTHOL HYDROXYANTHRANILIC ACID

2 amino 1 naphthol is not carcinogenic while its free or unconjugated form is carcinogenic McDonald[2] demonstrated that the carcinogen 2 amino 1 naphthol must have actual contact with the bladder epithelium to produce the cancer Boyland[3, 4] found that in the urine of patients with cancer of the bladder there is an increase of both the enzyme glucuronidase and of the deconjugated breakdown substances that it produces from tryptophan metabolism This was so even after the tumor had been removed Boyland[5] also showed that when given alone a certain carcinogen produced cancer in animals at sites other than the bladder but when tryptophan was given with this carcinogen then most of the animals developed carcinoma of the bladder Thus it was thought that in some people an enzyme in the kidney or urine acts on a safe or conjugated product of tryptophan to produce a deconjugated or free amino phenol which given sufficient time to act on the bladder mucosa would produce bladder cancer

## METHOD

The endogenous or urinary breakdown products which we used were synthesized by Professor Boyland and were 3 hydroxyanthranilic acid 3 hydroxykynurenine 3 hydroxyacetophenone xanthurenic acid and kynurenic acid A 20 per cent mixture of these substances was made up into 10 mg pellets using cholesterol as a base 10 mg pellets of 100 per cent cholesterol were used as a negative control and similar pellets of a 20 per cent mixture of 2 amino 1 naphthol and cholesterol were used as a positive control

These pellets were then placed into the bladder of mice and left there for 32 wks

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of papilloma-bearing bladders with skin grafts.[4,5] They removed the mucosa of the normal canine bladder and resurfaced it with thick split grafts of skin. Although the grafts were successful as evidenced by continued hair growth and corroborated by histologic section, pyuria, encrustations, calculi and bladder contracture developed. These complications have not been encountered in this series of experiments.

The results of canine vesical denudation encourage its possible application to the human with multiple recurrent papillomata or multiple papillomatosis of the urinary bladder. The procedure will aim at the extirpation of all papillomata together with the entire tumor-prone mucosa. Whether upper urinary tract dilatation and reflux will occur in the human remains to be seen. However, in the dog these complications are not serious and tend to disappear. They can be prevented by leaving a small rim of mucosa around the ureteral orifices, but this defeats the purpose of the operation, namely, the removal of all vesical epithelium with its tumor potential. It is hoped that newly proliferated uroepithelium derived from the urethra and ureters, where the incidence of papillary growths is statistically low, will prove more resistant to neoplasia.

## SUMMARY

1. A technique of total mucosal denudation of the canine bladder has been described.

2. The procedure is well tolerated by the dog.

3. The urinary bladder is completely re-epithelialized in 16 wks.

4. Upper urinary tract dilatation and reflux are transitory sequelae.

5. The results of this study suggest its application to the human with multiple papillomatosis or multiple recurrent papillomata of the bladder.

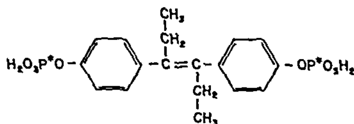
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# DISTRIBUTION AND METABOLISM STUDIES OF $P_{32}$ LABELLED DIETHYLSTILBESTROL DIPHOSPHATE*

LESTER PERSKY, FREDERICK J. BONTE AND JACK S. KROHMER

One of the aims of the chemotherapy of cancer is the differential localization of cytotoxic substances at the site of the neoplasm. A relatively new estrogen, diethylstilbestrol diphosphate (stilfostrol), which has been used with moderate success in cases of far advanced carcinoma of the prostate,[1] because of the nature of its chemical structure (Fig 1) suggested the possibility of achieving this therapeutic goal by the substitution of radioactive phosphorus ($P_{32}$) for the non-radioactive phosphorus in its molecule. The likelihood that the acid phosphatase of the prostate might selectively hydrolyze the drug had occurred to earlier workers[2,3] who were hoping to depress prostatic cancer by the possible direct action of estrogen locally in high concentrations. The incorporation of a radioactive isotope into the molecule therefore seemed to offer a potentially double-barrelled therapeutic tool. The clinical observation that burning perineal pain rapidly attended the intravenous administration of diethylstilbestrol diphosphate offered some hope that this hydrolysis was occurring. Even though it has been noted that malignant prostatic tissue is relatively low in acid phosphatase,[4] it seemed worthwhile to do distribution and excretion studies in a series of experimental animals to test the validity of the hypothesis that we might successfully secure cancerocidal radiation doses in carcinoma of the prostate and its metastases. This same hypothesis had occurred independently to a group of British workers in a consideration of the effect of prostatic acid phosphatase on the drug *in vitro*.[5]




$P^{32}$ LABELLED DIETHYLSTILBESTROL DIPHOSPHATE

Fig 1. Molecular structure of diethylstilbestrol diphosphate showing position of $P_{32}$.

## METHOD

The dog was utilized as the experimental animal because of the higher levels of acid phosphatase present in its prostate.[6] Two series of animals were utilized. The control group consisted of dogs which received inorganic $P_{32}$ as the phosphate ion intravenously. The experimental group received diethylstilbestrol diphosphate with substituted $P_{32}$. Because of the limited

*From the Department of Surgery, Urological Service and the Department of Radiology, Western Reserve University and the University Hospital, Cleveland, Ohio. Supported in part by the Miles Ames Research Laboratories, Elkhart, Indiana and conducted in part with facilities made available through contract number W 31 109 ENG 78 between the United States Atomic Energy Commission and Western Reserve University.

The mice, approximately 3 mo old and weighing 30 gm were anesthetized with an ether saturated cotton wool plug in a test tube then pinned onto a cork board and a transperitoneal cystotomy was performed using fine instruments

A small opening was made in the dome of the bladder the pellet inserted and the opening closed with a simple ligature The abdominal muscles and skin were brought together with a suture in each

After 32 wks the mice were sacrificed and autopsied In order to facilitate the histological interpretation of the bladder findings the bladders were distended with a 10 per cent solution of formaldehyde injected perurethrally They were then placed in 10 per cent solution of formaldehyde until fixed and then cut in the sagittal plane to produce a left and a right half of the bladder and each of these was examined histologically

## RESULTS

Forty pellets of each of the substances were implanted Just over one half of the mice died during the experiment All the mice showed chronic cystitis but only the 3 hydroxyanthranilic acid ones showed epithelial changes Seven of the 19 survivors implanted with this substance showed marked epithelial changes consisting of several layers of cell nests as down growths of the epithelium These cells were atypical with a pale cytoplasm There was no infiltration In the same bladders there were also small papillary lesions containing epithelial downgrowths

Eight mice had a cast calculus of the bladder but none of these showed epithelial hyperplasia and these came from all groups

## CONCLUSIONS

3 hydroxyanthranilic acid an unconjugated amino-phenol and a metabolite of tryptophan produced epithelial hyperplasia with downgrowths of cell nests while the other test substances and cholesterol produced only inflammatory changes Of the substances tested this one 3 hydroxyanthranilic acid most resembles the industrial carcinogens in chemical structure

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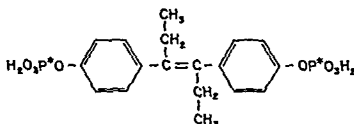



Fig 1 Molecular structure of diethylstilbestrol diphosphate showing position of $P_{32}$

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supply of the labelled drug in order to give adequate tracer doses only 5 animals were employed in each group Two millicuries were used in each animal in both the control and experimental group

After the intravenous injection of both materials samples of blood were drawn at 15 min 30 min 1 hr 3 hr 24 hr 72 hr and after 1 wk The dogs were sacrificed at ½ hr 1 hr, 3 hr 72 hr and after 1 wk in each group Specimens were taken of prostate rib muscle liver spleen and kidney in each animal In dogs surviving for 24 hr or more urines were collected daily

All counts were made on dried or ashed samples using a thin end window Geiger counter The counts were corrected for background and were compared to an aliquot of the original dose The counts were then expressed as a percentage of initial dose per gram based upon wet sample weight

RESULTS

Blood levels following injection of both labelled stilfostrol and inorganic $P_{32}$ are seen in Figure 2 The percentage of the initial dose is somewhat higher for the stilfostrol than for the phosphorus alone However, the slope of the curves are in general very similar The results of counting of the tissues are represented in Table 1 In the interest of brevity and clarity only the figures obtained after 1 day and after 1 wk are listed In general, the earlier figures were very similar to the 24 hr percentages except in the case of the prostate and the liver in which higher percentages were found at ½ hr, 1 hr and 3 hr

In the case of the prostate using labelled stilfostrol there was a definite increase in the percentage of the initial dose per gram found after 1 wk as compared to the 24 hr period This was not true for the inorganic $P_{32}$ which fell by approximately 50 per cent After 1 wk the rib and spleen analysis remained the same as the first day analysis for the stilfostrol but fell markedly for the $P_{32}$ alone The liver and kidney analysis fell approximately 50 per cent in both cases but the stilfostrol started at levels twice as great in both instances Muscle at the end of the week showed no deviation using inorganic $P_{32}$ but rose by 50 per cent with the stilfostrol $P_{32}$ The urinary concentration of the $P_{32}$ in the stilbestrol group was approxi

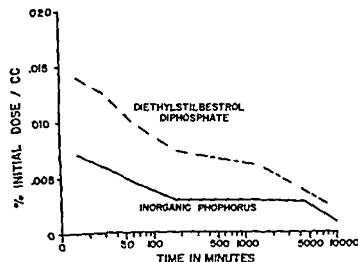



Fig 2 Disappearance curves of diethylstilbestrol diphosphate ($P_{32}$) and inorganic $P_{32}$ from blood

*Table 1 Percentage per gram of initial dose of diethylstilbestrol diphosphate labelled with $P_{32}$ and inorganic $P_{32}$ found on analysis of various tissues after one day and one week*

| | DAY | | WEEK | |
|---|---|---|---|---|
| | STILFOSTROL | $P_{32}$ | STILFOSTROL | $P_{32}$ |
| Prostate | 0 008 | 0 009 | 0 0128 | 0 005 |
| Rib | 0 008 | 0 015 | 0 009 | 0 003 |
| Spleen | 0 008 | 0 008 | 0 010 | 0 005 |
| Liver | 0 028 | 0 014 | 0 015 | 0 007 |
| Kidney | 0 026 | 0 014 | 0 014 | 0 006 |
| Muscle | 0 004 | 0 004 | 0 008 | 0 003 |

mately double that of the inorganic $P_{32}$ and was approximately 1 5 per cent of the total dose per day *following the first 24 hr*

DISCUSSION

The lower percentages of the initial dose found both early and late in our study is discouraging in terms of the therapeutic value of attaching radioactive phosphorus to the estrogen moiety of stilfostrol The rapid fall from the blood stream and the high concentration found early in the urine suggests that the phosphorus fraction can be expected to accomplish little in a cancerocidal sense The diffuseness of its distribution similarly points to a failure of this aim The counts found in the prostate after 1 wk suggests however that there is slight localization possibly due to the isotope still attached to the stilbestrol fraction The failure to attain really high levels initially may be due to the rapid diffusion of the phosphorus into the large body pool or may be a function of the effect of acid phosphatase present throughout the body The former impression is corroborated by the higher immediate counts found in the gland In general however our studies confirm the impression of Marberger[7] that there was little localization of this estrogen in prostatic tissue We will not completely eliminate however any cancerocidal effect until the labelled compound has been assayed clinically since tumor may handle the drug in a different fashion

The percentage of radioactivity still present in the rib after 1 wk *is interesting* but also discouragingly low If the level of radioactivity had been higher we would have viewed with more optimism the administration of the drug to cases of wide spread bony metastatic disease *responding to estrogenic* therapy The failure to fall as did inorganic $P_{32}$ may again be the result of that portion of the dose which remained unhydrolyzed The initial high counts found in the liver are probably due *in part to the liver's* generalized role of detoxification and conjugation The elevated renal counts at the start on the other hand are probably due to the renal clearance of the estrogen also still unhydrolyzed The increased counts in the muscle after 1 wk further illustrates the diffuseness of *the distribution*

Because of the small number of animals utilized and the limited amount of material available our figures and results cannot be analyzed statistically It is possible that the acid *phosphatase is not* a specific enough enzyme for this type of treatment It may be necessary to explore the efficacy of other isotopes in the place of the $P_{32}$ which may too rapidly gain equi

supply of the labelled drug in order to give adequate tracer doses, only 5 animals were employed in each group Two millicuries were used in each animal in both the control and experimental group

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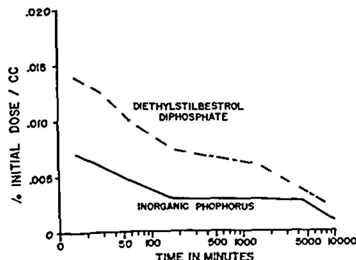



Fig 2 Disappearance curves of diethylstilbestrol diphosphate ($P_{32}$) and inorganic $P_{32}$ from blood

in 1948 by a more extensive study.[2] All the reports of such examinations of New York Hospital patients between August 1945 and June 1951 have been reviewed. Follow-up data for 5 yr are available in the cases of all but 1 of the 176 patients who had cancer cells found in their urine. Because our objective was to determine the reliability of the reports indicating cancer present rather than to evaluate the sensitivity of the test, no follow-up was pursued in the other cases. The first 200 urine specimens studied from before August 1945 were not included as it was felt that during that early period the techniques and diagnostic criteria might be considered in the formative stages rather than based on extensive clinical applications.

During the period selected 3107 patients had 9216 reports of urinary sediment examinations or 2.93 studies per patient. On a few occasions several specimens gathered from a patient over a 1 or 2 day period resulted in a single report. The specimens were prepared and studied by the staff of the George N. Papanicolaou Cytology Laboratory in the Department of Anatomy. The specimens were classified as follows:

Class 0—No classification possible (too few cells or inadequate preparation)

Class 1—No evidence of malignant neoplasm found

Class 2—Atypical cells present but no evidence of a malignant neoplasm

Class 3—Cells present giving a suspicion of a malignant neoplasm

Class 4—Fairly conclusive evidence of a malignant neoplasm

Class 5—Conclusive evidence of a malignant neoplasm

In the first few years of these studies occasional specimens were reported in overlapping categories such as 3–4 or 4–5. When these were used for the purposes of this review the lower of the 2 categories was taken. Specimens were submitted by all departments of the hospital by whatever indications the individual physician wished to use. The series was not a cross section of the general population nor necessarily of a general urological or cancer practice.

Of the 9216 cytologic studies there were 442 (4.6 per cent) positive (Class 4 or 5). Of these 442, 175 (39 per cent) were Class 4 and 267 (61 per cent) were Class 5. Of the 3107 patients who submitted urine for study, 176 (5.7 per cent) had reports indicating the presence of cancer cells in the urine.

The sources of the exfoliated cancer cells in the urine are listed in Table 1.

The 11 unexplained reports of cancer cells in the urine concerned 8 patients. These 11 reports represent 2.4 per cent of all the positive reports in 8 patients or 4.6 per cent of the 176 patients with positive reports. It can be noted that the majority (72.7 per cent) of the unexplained reports were of Class 4. The follow-up is practically absent in 1 case (NYH No. 477212).

There were numerous examples of patients with positive reports and without immediate urological evidence of cancer who on subsequent examinations have proven to have genitourinary cancer. The changes in circumstances from an unexplained report of cancer cells in the urine to proven genitourinary cancer has been so frequent with the passage of time that it is mandatory to follow indefinitely any such unexplained finding.

The positive smears that were unexplained in this group of old cases were recently reviewed independently by 2 staff cytologists. Perhaps the experience of an additional 5 or more years in evaluating these smears and improvements in criteria would affect the present day classification. All but

librium with the general body pool The possibility also still exists that unattached radioactive isotopes may specifically localize in prostatic tissue as suggested by Gunn and Gould[8] using $Zn^{65}$ in the rat Finally, since our current studies have not eliminated the possibility that the estrogen itself is organ specific we hope to continue this investigation with $C^{14}$ labelled stilbestrols which will give us some clue as to the fate of the estrogenic portion itself


## SUMMARY

Preliminary experiments are described in which diethylstilbestrol diphos phate labelled with radioactive phosphorus was employed to determine the possible hydrolysis of this compound by prostatic acid phosphatase These initial studies suggest only minimal localization of the isotope in the pros tate as compared with other tissues The slightly greater uptake however by prostate and bone suggests that further exploration is indicated with this and similar compounds and other isotopes


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# THE RELIABILITY OF THE PAPANICOLAOU TECHNIQUE WHEN CANCER CELLS ARE FOUND IN THE URINE*


SAMUEL I ROLAND AND VICTOR F MARSHALL


Early in 1945 cytologic studies of the urine were made available by the Papanicolaou Cytology Laboratory for the use of all departments of the New York Hospital Cornell Medical Center A preliminary report of these studies was made by Papanicolaou and Marshall[1] in 1945 and was followed


*From the Department of Surgery (Urology) Cornell University Medical College and the Department of Surgery (Urology) James Buchanan Brady Foundation of The New York Hospital


Aided by the staff of the Papanicolaou Cytology Laboratory and partic ularly by Dr John F Seybolt of the New York Hospital—Cornell Medical Center

was done in this entire series of cases. The original classifications are the only ones used elsewhere in this review.

## DISCUSSION

There is no doubt that occasional inexplicable or false positive reports of cancer cells in the urine will be made. Several such instances have been reported in recent medical literature[6, 7, 8] As in any laboratory aid to clinical evaluation this error, however small, must be kept in mind. The finding of a 4.6 per cent unexplained positive urine cytology in 8 of 175 patients with Class 4 or 5 urine reports has been sufficiently small to make us highly suspicious that a patient with such cells in the urine harbors a urinary cancer which may not have made itself evident in the usually recognizable forms. Five of these 8 patients were followed for over 5 yr. Studies of exfoliated cells in the urine have been a valuable part of the investigation of patients for urinary cancer, especially those cancers arising from the transitional epithelium. Cancers of the renal parenchyma and small prostatic cancers were not often detected by this method, but when cancer cells were found in such cases the report was reliable. Cytologic studies did occasionally push back the point of recognition of such disease and make possible its early diagnosis. It should be emphasized that these examinations were made by experienced cytologists.

## SUMMARY

1 9216 reports of the examination of urinary sediment smears from 3107 patients were reviewed.

2 442, 4.6 per cent of the 9216 reports indicated the presence of malignant neoplastic cells (Class 4 or 5).

3 These 442 reports were found in the cases of 176 patients, 5.7 per cent of the 3107 patients who contributed specimens.

4 Cancer has not been demonstrated in 8 (4.6 per cent) of these 176 patients. Five of the 8 patients have been followed for 5 or more yr, 2 have died without evidence of a urinary cancer prior to the elapse of 5 yr, and 1 patient has disappeared from follow up after 8 mo.

5 A number of impressive cases were found in which the Papanicolaou method provided very early evidence of cancer—before careful urological examination did. Some of these cases are briefly described.

## CONCLUSION

When it was reported that cancer cells were present in the urine the patient had cancer in the urinary tract in at least 95 per cent of the cases. A positive report which is unexplained warrants the following of the patient for an indefinite period of time.

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*Table 1 Sources of the Exfoliated Cancer Cells in the Urine of 176 Patients*

| 1 CASES PROVEN BY BIOPSY | NUMBER OF REPORTS AND CLASS 4 | 5 | NUMBER OF PATIENTS |
|---|---|---|---|
| Renal carcinoma | | | |
| Pelvic | 4 | 11 | 6 |
| Parenchymal | 2 | 1 | 2 |
| Ureteral carcinoma | 0 | 1 | 1 |
| Bladder carcinoma | 120 | 215 | 123 |
| Bladder leiomyosarcoma | 1 | 0 | 1 |
| Bladder papilloma (Grade 1 carcinoma in some classifications) | 7 | 7 | 6 |
| Bladder papilloma with cystitis cystica | 3 | 1 | 1 |
| Prostatic carcinoma | 22 | 14 | 16 |
| Urethral carcinoma | 2 | 5 | 2 |
| Penile carcinoma | 0 | 1 | 1 |
| Penile melanosarcoma | 0 | 1 | 1 |
| Metastatic carcinoma to the urinary tract | | | |
| Adrenal to kidney | 1 | 0 | 1 |
| Stomach to kidney | 0 | 1 | 1 |
| Sigmoid to bladder | 0 | 1 | 1 |
| Ovaries to bladder | 0 | 1 | 1 |
| Rectum to bladder | 3 | 0 | 2 |
| Uterus to bladder | 1 | 0 | 1 |
| Pancreas with carcinomatosis | 1 | 0 | 1 |
| 2 CASES UNEXPLAINED BY BIOPSY | 8 | 3 | 8 |
| Total | 175 | 267 | 176 |

1 slide was available for review Table 2 is a review of the smears in the cases of inexplicably positive cytology of the urine

It can be seen that all but 2 patients had their smears re-classified so that cancer was not indicated with patients (NYH No s 602036 and 436517) retaining almost the same classification In only the last 2 cases did the two cytologists differ in each instance by only 1 class It should be noted that the reclassifications cited in Table 2 are the only occasions a reclassification

*Table 2 A Review of the Unexplained Class 4 and 5 Urine Papanicolaou Smear*

| NYH NO | DATE ORIGINALLY READ | ORIGINAL CLASSIFICATION | NEW CLASSIFICATION CYTOLOGISTS A | B |
|---|---|---|---|---|
| 436517 | 5 7 46 | 5 | 4 | 4 |
| 477212 | 6 2 47 | 4 | 3 | 3 |
| 221701 | 12 4 48 | 4 | 2 | 2 |
| | | 4 | 3 | 3 |
| | | 4 | 3 | 2 |
| 424492 | 3 6 49 | 4 | 2 | 2 |
| 565869 | 3 7 50 | 4 | 2 | 2 |
| 510953 | 4 7 50 | 4 | 2 | 2 |
| 591966 | 2 4 51 | 4 | 2 | 2 |
| 602036 | 6 5 51 | 5 | 5 | 4 |
| | 6 9 51 | 5 | 3 | 2 |

intrarenal vessels (the interlobular and arcuate arteries) consisting of narrowing irregularity and decreased number of vessels These changes were consistent with chronic glomerulonephritis or arteriolonephrosclerosis *Eight other* hypertensive patients who were known to have parenchymal renal disease were included in the investigation The following diseases were represented chronic pyelonephritis (3) bilateral hydronephrosis (1) congenital hypoplasia (1) radiation nephritis and Wilms tumor (1) traumatic pericapsular hematoma (1) and nephrocalcinosis (1) Three patients were considered to have aortograms inadequate for diagnostic purposes

It is interesting to note that among the 53 *nonhypertensive patients* studied during the same period *seven* other patients were found to have nonobstructive renal artery disease Four patients had congenital aneurysm 1 had a large arteriovenous aneurysm and 2 had calcified arteriosclerotic plaques within the wall of the renal artery without encroachment of the lumen

*Table 1 Pathology grade of hypertensive disease method and result of treatment of 16 patients with renal artery lesions found among 67 hypertensive patients studied by aortography January 1955, through August 1956*

| PATHOLOGY | HYPERTENSIVE DISEASE | NUMBER OF PATIENTS | TREATMENT | RESULT |
|---|---|---|---|---|
| Arteriosclerotic plaques in main artery | Malignant | 7 | Nephrectomy<br>Homograft<br>Antipressor drugs | 3 Cured<br>1 Dead postop hemorrhage<br>3 {2 dead, 1 unchanged} |
| | Benign | 3 | Antipressor drugs | 2 unchanged<br>1 dead |
| Arteriosclerosis of branches | Malignant | 1 | Nephrectomy | Improved |
| | Severe | 1 | Attempted endarterectomy of solitary kidney | Dead—renal failure |
| Fibrous intimal proliferation | Severe | 1 | Bilateral arterial homograft | Cured |
| Thrombosis of main artery | Malignant | 2 | Nephrectomy | 2 {1 cured, 1 improved} |
| Stenosis with microaneurysms | Benign | 1 | Nephrectomy | Cured |

**Pathology** A considerable variety of renal artery lesions were found in the 16 hypertensive patients Chief among these are thick obstructive arteriosclerotic plaques occurring in various locations along the renal artery Ten patients were recognized as having this type of lesion and 3 of these demonstrated poststenotic or jet dilatation distal to the plaque[7] Arteriosclerosis involving the branches of the renal artery was found in 2 patients One patient had fibrous intimal proliferation of both renal arteries[9] Two patients had thrombosis of the main renal artery with viability of part of the kidney assured because of an aberrant arterial circuit The last patient had

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5 Weyrauch Henry M and Presti Joseph C Papanicolaou examination of urine in diagnosis of urinary cancer False positives in diagnosis of renal neoplasms J Urol Balt *75* 551 557 1956
6 Chute R and Williams D W Experiences with stained smears of cells exfoliated in urine in diagnosis of carcinoma of genito urinary system J Urol Balt *59* 604 618 1948
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## REVERSIBLE HYPERTENSION DUE TO RENAL ARTERY DISEASE*

E F POUTASSE W J ENGEL, AND H P DUSTAN

Over 20 years ago Goldblatt showed that constriction of the renal artery of the dog produced hypertension The clinical counterpart of this hyper tension is now recognized but there are comparatively few well documented case reports[1] From 1950 through 1954 we observed 6 patients who had either unilateral or bilateral obstructive lesions of the renal artery as the primary cause of hypertensive vascular disease The 2 with bilateral lesions have been previously reported[2] The 4 other patients had unilateral lesions and had reversal of their hypertensive state to normal by nephrectomy All of these 6 patients had translumbar aortography with demonstration of the renal artery pathology

Since 1955 aortography has been used on a much wider scale in the in vestigation of hypertension and the purpose of this paper is to present the results of this investigation

Aortography was not employed routinely in the evaluation of patients with hypertension but was used in patients without family history of essen tial hypertension in the young (below the age of 35) without renal par enchymal disease in the elderly with accelerated hypertension and in those with a history suggesting the possibility of thrombosis or embolism of the renal artery

**Aortography in the Investigation of Hypertension** From January 1955 through August 1956 109 patients were studied by aortography by the Urologic Service of these 67 were patients with all grades of hypertension from benign to the malignant hypertensive syndrome *Sixteen* of the hyper tensive patients (24 per cent) were found to have demonstrable obstructive lesions of the main renal artery or its immediate branches 14 had unilateral and 2 had bilateral lesions These obstructive lesions were considered to be instrumental in the onset and maintenance of the hypertensive state

*Eight* others of the hypertensive patients had recognizable changes in the

*From the Department of Urology and the Division of Research The Cleveland Clinic Foundation and The Frank E Bunts Educational Institute Cleveland Ohio

intrarenal vessels (the interlobular and arcuate arteries) consisting of narrowing, irregularity and decreased number of vessels. These changes were consistent with chronic glomerulonephritis or arteriolonephrosclerosis. *Eight other* hypertensive patients who were known to have parenchymal renal disease were included in the investigation. The following diseases were represented: chronic pyelonephritis (3), bilateral hydronephrosis (1), congenital hypoplasia (1), radiation nephritis and Wilms tumor (1), traumatic pericapsular hematoma (1) and nephrocalcinosis (1). Three patients were considered *to* have aortograms inadequate for diagnostic purposes.

It is interesting to note that among the 53 *nonhypertensive patients* studied during the same period *seven* other patients were found to have nonobstructive renal artery disease. *Four patients had congenital aneurysm*, 1 had a large arteriovenous aneurysm, and 2 had calcified arteriosclerotic plaques within the wall of the renal artery without encroachment of the lumen.

*Table 1. Pathology, grade of hypertensive disease, method and result of treatment of 16 patients with renal artery lesions found among 67 hypertensive patients studied by aortography, January 1955 through August 1956*

| PATHOLOGY | HYPERTENSIVE DISEASE | NUMBER OF PATIENTS | TREATMENT | RESULT |
|---|---|---|---|---|
| Arteriosclerotic plaques in main artery | Malignant | 7 | Nephrectomy<br>Homograft<br>Antipressor drugs | 3 Cured<br>1 Dead, postop hemorrhage<br>3 {2 dead, 1 unchanged} |
| | Benign | 3 | Antipressor drugs | {2 unchanged, 1 dead} |
| Arteriosclerosis of branches | Malignant | 1 | Nephrectomy | Improved |
| | Severe | 1 | Attempted endarterectomy of solitary kidney | Dead—renal failure |
| Fibrous intimal proliferation | Severe | 1 | Bilateral arterial homograft | Cured |
| Thrombosis of main artery | Malignant | 2 | Nephrectomy | 2 {1 cured, 1 improved} |
| Stenosis with microaneurysms | Benign | 1 | Nephrectomy | Cured |

**Pathology.** A considerable variety of renal artery lesions were found in the 16 hypertensive patients. Chief among these are thick obstructive arteriosclerotic plaques occurring in various locations along the renal artery. Ten patients were recognized as having this type of lesion and 3 of these demonstrated poststenotic or jet dilatation distal to the plaque.[3] Arteriosclerosis involving the branches of the renal artery was found in 2 patients. One patient had fibrous intimal proliferation of both renal arteries.[2] Two patients had thrombosis of the main renal artery with viability of part of the kidney assured because of an aberrant arterial circuit. The last patient had

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and filling of the arteries themselves The procedure is done under local anesthesia The chief pitfalls in the diagnosis of renal artery lesions are the misinterpretation of an inadequate aortogram and the failure to appreciate the significance of even minor variations from normal in the renal artery or in the comparative sizes of the kidneys

## CONCLUSIONS

Obstructive lesions of the renal artery have been found to be associated with hypertensive vascular disease presumably because of changes in the renal circulation This hypertension may be benign or accompanied by a necrotizing vascular disease (malignant hypertension) Proper treatment either by nephrectomy or arterial homograft, can cause remission of the hypertension

Aortography is at present the most important means of diagnosis of renal artery disease

Sixty seven hypertensive patients were studied by aortography between January 1955 and August 1956 16 of these patients were found to have renal artery obstructive lesions This does not represent the true incidence of renal artery lesions in hypertensive patients Eleven patients were treated surgically and, in 8 hypertensive disease cured or improved

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partial stenosis with multiple congenital microaneurysms of the renal artery and 2 of its branches

**Treatment** Of the 16 patients with renal artery obstructive lesions 11 underwent surgical treatment Seven had nephrectomy and thus far have had their hypertensive vascular disease either cured or improved Of these 7 6 had malignant hypertension Four had normal blood pressure after nephrectomy in 2 elderly patients the malignant syndrome disappeared and diastolic hypertension was controlled although systolic hypertension persisted One patient underwent bilateral renal artery homografting to restore normal circulation to both kidneys with restoration of his blood pressure to normal[2] One 30 yr old man with malignant hypertension and incomplete obstruction of one renal artery by 2 plaques died of hemorrhage 2 wks following attempted renal artery homograft One hypertensive patient had excision of a congenital aneurysm with repair of the renal artery *but the opposite renal artery has an arteriosclerotic plaque with distal jet* dilatation She is still under care at the present time The patient with microaneurysms and stenosis of one renal artery has had normal blood pressure since nephrectomy She had had asymptomatic hypertension for 3 yr The eleventh patient had a solitary atrophic kidney and was found to be incurable by present surgical techniques because of arteriosclerosis with incomplete obstruction of all branches of the renal artery He has since died of renal failure

Five patients with renal artery obstructive lesions and hypertension did not undergo surgery Two of these patients had far advanced malignant hypertension and died within a short time of progressive disease They both had obstructing plaques at the orifice of one renal artery with a small kidney One patient with a small arteriosclerotic plaque causing obstruction of one renal artery has failed to return for any treatment One patient with bilateral obstructing lesions of the renal artery and severe hypertension died of widespread arteriosclerosis and massive cerebral thrombosis The fifth patient is under antipressor drug therapy at present

**Diagnosis of Renal Artery Disease** Intravenous urography should be performed in all non azotemic hypertensive patients Unfortunately this study provides no consistent signs that would lead one to suspect the possibility of renal artery obstructive disease There may be slight reduction in renal mass or slight reduction in function of the involved kidney but several patients with serious obstructive arterial lesions had what appeared to be a normal intravenous urogram One patient with thrombosis of the main artery for example was considered to have essentially normal excretory renal function by urography probably because blood was still getting into the kidney through a large branch proximal to the thrombosis

Comparison of the urine samples from each kidney in hypertensive patients *is of considerable value Kidneys with constricted arteries may show* impairment of renal function as shown by the reduced concentration of substances in the urine as well as reduction in urine volume

Translumbar aortography is the only means of demonstrating the presence of renal artery defects The technique has been previously described[4] We have found that in patients with extremely elevated blood pressure it is helpful to use vasodepressor drugs to reduce the blood pressure and thereby obtain better compression of the aorta distal to the renal arteries

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